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THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS
AND OTHER ANALYTICAL CHEMISTS

A JOINT meeting of the Society with the Food Group of the Society of Chemical Industry was held at 6.30 p.m., on Wednesday, December 4th, 1946, at the Chemical Society's Rooms, Burlington House, Piccadilly, London, W.1. The general subject of the meeting was "The Application of Statistical Methods to Food Problems."

At the request of the Chairman of the Food Group, Mr. T. Rendle, the Chair was taken by the President of the Society, Dr. G. W. Monier-Williams. After the minutes of the last meeting of the Society had been read and confirmed the meeting proceeded to its main business and the following papers were presented and discussed:—Introductory Address: "The Inevitability of Statistics," by D. J. Finney, M.A.; "The Use of Statistical Analysis in Research in Food Canning," by W. B. Adam, M.A., F.R.I.C.; "The Evaluation of the Nutritive Value of Animal Feeding Stuffs," by Dr. K. L. Blaxter (read by Dr. W. M. Allcroft); "Application of Statistical Methods in Calculating Proportions of Ingredients in Certain Food Products," by E. H. Steiner, B.Sc., A.R.I.C.

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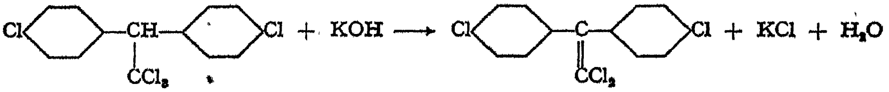
The Estimation of 2:2-Bis-(*p*-chlorophenyl)-1:1:1-trichloroethane (*p,p'*-DDT) by Methods depending on its Dehydrohalogenation

BY R. L. WAIN AND A. E. MARTIN

IT was first demonstrated by Zeidler[1] that 2:2-bis-(*p*-chlorophenyl)-1:1:1-trichloroethane (*p,p'*-DDT), when heated under reflux with alcoholic potassium hydroxide, loses one molecule of hydrogen chloride to form 2:2-bis-(*p*-chlorophenyl)-1:1-dichloroethylene (DDX), thus,



and since the use of DDT as an insecticide this reaction has been utilised by a number of workers for its estimation. Providing that the dehydrohalogenation occurs quantitatively as in the equation above and that the alkali has no further action on the DDX produced, the DDT can be estimated indirectly either by acidimetric titration of the alkali consumed or by determination of the chloride ion formed. The former method, although rapid, is more susceptible to analytical error and has not been greatly employed. The latter procedure has been used by Gunther[2] and others.[3,4,5,6,7]

Both methods were examined in 1943 by one of the present writers, using pure *p,p'*DDT. With both, high results were frequently obtained, indicating either that hydrolysis of the substance had proceeded too far or that one or more atoms of chlorine were being displaced from DDX by the action of the alcoholic alkali. To test this latter possibility, DDX was heated under reflux for varying times with *N* alcoholic potassium hydroxide. Under these conditions, the substance was apparently stable. When 25 per cent. alcoholic potash solution was used, however, the mixture gave a strongly positive reaction for chloride after four

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hours, and after sixteen hours bis-(*p*-chlorophenyl)-acetic acid was readily isolated from the mixture. This acid, since described by Grummitt *et al.*,[8] was also prepared in quantity by heating either *p,p'*-DDT or DDX with alcoholic sodium ethoxide in a sealed tube (*cf.* Fritsch and Feldman[9]). Finally it was isolated from the products obtained by heating *p,p'*-DDT with *N* alcoholic potassium hydroxide for 30 minutes under reflux.

These experiments show that, when heated under reflux with alcoholic alkali, *p,p'*-DDT can yield more than one molecule of hydrogen chloride per molecule, and they thus afford an explanation of the high analytical results already noted.

Commercial samples of DDT contain about fourteen substances,[10] the chief of which are *p,p'*-DDT (63 to 77 per cent.), 1-trichloro-2-*o*-chlorophenyl-2-*p*-chlorophenylethane (*o,p'*DDT) (8 to 21 per cent.), 1:1-dichloro-2:2-bis-(*p*-chlorophenyl)-ethane (DDD) (0·3 to 4·0 per cent.), 2-trichloro-1-*o*-chlorophenylethyl-*p*-chlorobenzenesulphonate (0·4 to 1·85 per cent.) and *p*-chlorophenyl-trichloromethyl-carbinol (about 0·2 per cent.). Methods of estimation depending on dehydrochlorination applied to such a mixture would therefore not be expected to be reliable, quite apart from the considerations outlined above. For this reason the present work was restricted to an investigation of the behaviour of pure *p,p'*-DDT and DDX with alcoholic alkali under various conditions. The first method adopted involved the estimation of chloride ion by Volhard titration, by a procedure similar to that adopted by Gunther.[2] In addition, an acidimetric method was employed in which the loss of alkalinity in the ethanolic potassium hydroxide solution was determined directly by titration.

Materials—

p,p'-DDT—The commercial material, crystallised three times from alcohol. Colourless needles, m.p. 109° to 109·5° C.

DDX—Prepared from pure *p,p'*-DDT by the action of alcoholic potassium hydroxide. Crystallised three times from alcohol. M.p. 87° to 88° C.

Standard alcoholic potassium hydroxide solution—The pure solid was heated under reflux with redistilled absolute alcohol and the solution was cooled, filtered and standardised.

EXPERIMENTAL

ACTION OF BOILING ALCOHOLIC POTASSIUM HYDROXIDE SOLUTION ON *p,p'*-DDT

(*a*) *Modified Gunther procedure for the estimation of p,p'-DDT*—Weighed quantities of *p,p'*-DDT were introduced into a 250-ml. round-bottomed flask of the Quickfit type and 50 ml. of *N* alcoholic potassium hydroxide were added. A reflux condenser was fitted, and the mixture was heated over a naked flame. After boiling for 30 minutes, 25 ml. of water, 50 ml. of 2 *N* nitric acid and a further 25 ml. of water were added successively through the vertical condenser. The condenser was then detached and 3 ml. of redistilled nitrobenzene were added to the mixture and followed by 25 ml. of 0·1 *N* silver nitrate. After stoppering and shaking the flask to coagulate the silver chloride and coat the particles with a layer of nitrobenzene, the stopper was removed and rinsed into the flask with water. Three ml. of *N* ferric nitrate were added and the excess of silver was titrated with 0·05 *N* potassium thiocyanate. Blank estimations without DDT led to a small correction which was allowed for in all results. These results, calculated on the assumption that one molecule of DDT yields one molecule of hydrogen chloride under these conditions, are assembled in Table I.

(*b*) *Procedure with acidimetric titration ending*—Weighed quantities of *p,p'*-DDT were heated under reflux with 50 ml. of *N* alcoholic potassium hydroxide for 30 minutes as described above. The condenser and joint were then rinsed into the flask with water, 50 ml. of standard nitric acid were added and the mixture was titrated with 0·1 *N* sodium hydroxide, using phenolphthalein as indicator. The standard acid used was slightly stronger than the alcoholic alkali; this ensured a small titration of alkali for the blank determination, which was allowed for in each estimation.

The results, calculated as before, are given in Table I.

TABLE I

ESTIMATION OF *p,p'*-DDT

	Ending with Volhard titration							
Wt. of DDT taken, mg. ..	119·5	159·0	161·0	344·0	377·0	617·8	640·0	
Recovery, per cent. ..	104·2	103·8	105·3	105·3	105·9	102·1	101·0	
	Ending with acidimetric titration							
Wt. of DDT taken, mg. ..	118·9	201·9	239·8	312·4	470·5	617·2	805·0	1594·2
Recovery, per cent. ..	106·2	99·1	104·3	104·6	102·3	108·6	105·8	103·5

(*c*) *Effect of duration of boiling on dehydrohalogenation of p,p'-DDT*—Weighed quantities of *p,p'*-DDT were refluxed with 50 ml. of *N* alcoholic potassium hydroxide for measured times and the chloride ion present in the system was estimated as in (*a*). The results are given in Table II.

TABLE II

EFFECT OF DURATION OF BOILING ON DEHYDROHALOGENATION OF *p,p'*-DDT

Boiling period hours	Wt. of DDT taken mg.	Recovery %	Boiling period hours	Wt. of DDT taken mg.	Recovery %
0·25	501·2	102·6	4·0	503·5	102·8
,,	500·1	102·4	,,	498·2	105·2
0·5	499·0	102·5	8·0	499·4	105·5
,,	500·6	104·4	12·0	502·1	107·5
1·0	503·3	106·1	16·0	511·1	107·7
,,	499·5	104·1	24·0	500·0	107·9
2·0	497·3	103·2	48·0	499·4	111·1
,,	500·7	105·1			

DEHYDROHALOGENATION OF *p,p'*-DDT BY ALCOHOLIC ALKALI AT LOWER TEMPERATURES—

Twenty-five ml. lots of a solution of *p,p'*-DDT in alcohol were pipetted into 300-ml. flasks and 25-ml. quantities of standard (2 *N* or 0·2 *N*) alcoholic potassium hydroxide solution were added. The flasks were stoppered and allowed to stand at the temperature of the laboratory (18° to 18·5° C.) or in an incubator at 23° C. for measured times, after which the reaction was stopped by addition of excess of standard nitric acid. Chloride ion was then estimated in the mixtures by the procedure outlined above (p. 2). All results are given in Table III.

TABLE III

DEHYDROHALOGENATION OF *p,p'*-DDT AT LOWER TEMPERATURES

497·9 mg. of *p,p'*-DDT in 50 ml. of 0·996 *N* alcoholic KOH at 18° to 18·5° C.		495·0 mg. of *p,p'*-DDT in 50 ml. of 0·0986 *N* alcoholic KOH at 23° C.	
Time mins.	DDT recovered %	Time mins.	DDT recovered %
5	99·7	—	—
10	99·7	10	83·8
15	99·7	—	—
20	99·8	20	97·0
30	99·9	30	99·7
45	100·0	40	99·5
60	99·8	60	100·0
90	100·0	—	—
120	99·8	120	100·1
150	99·9	—	—
180	99·8	180	100·1

ACTION OF BOILING POTASSIUM HYDROXIDE SOLUTION ON DDX—

(i) *Effect of time on the decomposition of DDX by boiling alcoholic alkali*—A series of experiments as in (*c*) above were carried out with DDX, the percentage decomposition being calculated on the assumption that both non-nuclear chlorine atoms become displaced as chloride ion. Results are given in Table IV.

TABLE IV

EFFECT OF DURATION OF BOILING AND PRESENCE OF POTASSIUM CHLORIDE ON THE DECOMPOSITION OF DDX

Duration of boiling, hrs. . .	0·5	4·0	8·0	24·0	48·0
Decomposition of DDX, %:					
In absence of KCl . .	0·12 to <0·08	0·12	0·41	3·51	15·09
In presence of KCl . .	<0·08	0·6 to <0·08	0·35	1·71	5·67

The weight of DDX taken for each experiment was approximately 500 mg. (496·1 to 506·7)

(ii) *Influence of potassium chloride on the rate of decomposition of DDX by boiling alcoholic alkali*—A further series of experiments carried out as in (i) above but in presence of an approximately equimolecular quantity of potassium chloride gave the results shown in Table IV.

FORMATION OF BIS-(*p*-CHLOROPHENYL)-ACETIC ACID FROM *p,p'*-DDT AND FROM DDX—

(*a*) Eight g. of DDX were heated at 120° C. for 24 hours in a sealed Carius tube with alcoholic sodium ethoxide, prepared by dissolving 1·8 g. of sodium in 30 ml. of absolute alcohol. To the product 300 ml. of water were added, and the alcohol was then distilled off and the mixture extracted with ether. The extract, after removal of ether, gave 4·72 g. of solid having m.p. 72° to 79° C. After crystallisation from alcohol it melted at 83° to 87° C. This substance did not depress the melting-point of pure DDX and was therefore the unchanged starting material. The aqueous layer was freed from ether and filtered. On acidification the filtrate yielded 3·05 g. of a white crystalline solid having m.p. 164° to 166° C. This was crystallised twice from chloroform and obtained as colourless prisms, m.p. 166° C., which were shown by analysis to be bis-(*p*-chlorophenyl)-acetic acid. Yield: 33·8 per cent. (Found: C, 59·8%; H, 3·3%; equiv., 281·0. Calc. for $C_{14}H_{10}O_2Cl_2$: C, 59·8%; H, 3·6%; equiv., 281·0.) Grummitt *et al.*[8] give m.p. 163 to 164° C.

The following derivatives were prepared—

Ethyl bis-(*p*-chlorophenyl acetate) $(ClC_6H_4)_2CHCOOC_2H_5$.* Colourless prisms from alcohol, m.p. 89° C. (Found: C, 62·3%; H, 4·7%; $C_{16}H_{14}O_2Cl_2$ requires C, 62·2%; H, 4·6%.)

Bis-(p-*chlorophenyl*)*-acetamide* $(ClC_6H_4)_2CHCONH_2$. Colourless needles from methyl alcohol, m.p. 157° C. (Found: N, 4·9%; $C_{14}H_{11}ONCl_2$ requires N, 5·0%.)

Bis-(*p*-chlorophenyl)-acetanilide $(ClC_6H_4)_2CHCONHC_6H_5$.* Slender colourless needles from methyl alcohol, m.p. 208° to 209° C. (Found: N, 4·1%. $C_{20}H_{15}ONCl_2$ requires N 3·9%.)

(*b*) A similar experiment using 10 g. of *p,p'*-DDT, 2·6 g. of sodium and 45 ml. of alcohol, heated in a sealed tube at 120° C. for 24 hours, yielded 3·35 g. of bis-(*p*-chlorophenyl)-acetic acid of m.p. 164° to 165° C (42·2% of theoretical) and 4·7 g. of unchanged DDX.

(*c*) A solution of 4 g. of DDX in 100 ml. of absolute alcohol containing 25 g. of potassium hydroxide was heated under reflux. The solution gradually became cloudy and, after 4 hours a small portion gave a strongly positive reaction for chloride. After 16 hours the mixture was diluted with water, alcohol was distilled off and the aqueous solution extracted with ether. The extract yielded unchanged DDX, having m.p. 83° to 86° C. after one crystallisation from methyl alcohol. The aqueous solution was freed from ether, filtered and acidified. The white solid that separated was crystallised successively from methyl alcohol and twice from chloroform, yielding colourless prisms, m.p. 166° C., which did not depress the melting-point of the analysed specimen of bis-(*p*-chlorophenyl)-acetic acid.

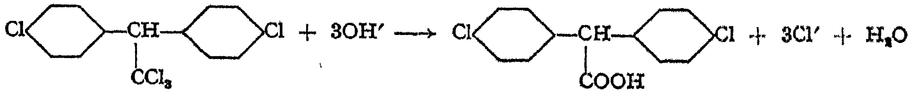
(*d*) An experiment carried out exactly as in (*c*), but on 4 g. of *p,p'*-DDT, gave 0·32 g. of the same acid.

(*e*) Five g. of *p,p'*-DDT boiled under reflux with *N* alcoholic potassium hydroxide gave a small yield (about 15 mg.) of bis-(*p*-chlorophenyl)-acetic acid, the identity of which was proved by melting and mixed melting points.

DISCUSSION

Methods of analysis of *p,p'*-DDT based on the determination of chloride or consumed alkali after boiling under reflux with *N* alcoholic potassium hydroxide gave results significantly higher than the theoretical value (Tables I and II). Gunther,[2] however, who used similar conditions and method of estimating chloride ion, obtained not more than 101·0 per cent. recovery in his experiments with pure *p,p'*-DDT, and in two of his seven recorded estimations less than 100 per cent. recovery was obtained. Nevertheless, the treatment he recommended, *viz.*, boiling under reflux with *N* alcoholic potassium hydroxide for 30 minutes, would appear to be unnecessarily drastic, for, as Brand and Busse-Sundermann[11] have shown, 5 g. of *p,p'*-DDT become dehydrohalogenated completely within three minutes on heating with 50 ml. of 0·5 *N* alcoholic alkali under reflux (see also Table II).

That *p,p'*-DDT becomes partially converted to bis-(*p*-chlorophenyl)-acetic acid by boiling with alcoholic potash, has been demonstrated. If it is assumed that DDX is not an intermediate product, this reaction may be represented as follows:



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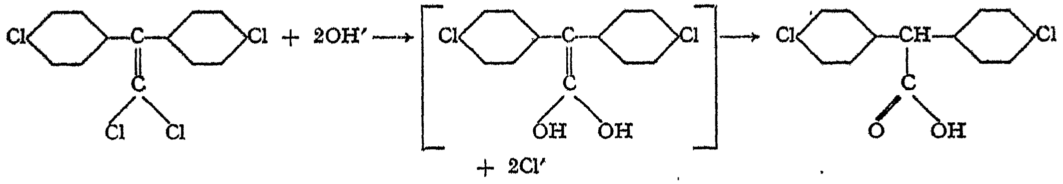
[*] Since this work was completed the ethyl ester and anilide have been described by Gätzi and Stammbach (*Helv. Chim. Acta*, 1946, **29**, 563) who give melting-points 89° to 90° C. and 213° to 214° C. respectively.

and it will be noted that three atoms of chlorine per molecule of DDT are liberated as chloride ion. A similar decomposition has recently been shown to occur with *o,p'*-DDT. Thus, although this substance is dehydrochlorinated only 1/67th as fast as the *p,p'*-isomer,[6] on boiling with alcoholic alkali for 30 minutes, Cristol and Haller[12] showed that the former compound undergoes a decomposition in which 4 per cent. of (*o*-chlorophenyl)-(*p*-chlorophenyl)-acetic acid is produced. The same workers, however, demonstrated that this amount is not further increased by longer heating and also that the ethylene derivative, 2-(*o*-chlorophenyl)-(*p*-chlorophenyl)-1:1-dichloroethylene, is stable to boiling alcoholic alkali. For these reasons it was concluded that the ethylene derivative is not an intermediate product in the formation of the substituted acetic acid from *o,p'*-DDT.

In the present work similar results were noted. Thus, when *p,p'*-DDT was boiled under reflux with *N* alcoholic potassium hydroxide for 30 minutes, approximately 1·04 equivalents of chloride ion per mole of DDT were liberated, an amount which increased only slightly on continuing the boiling for longer periods up to 24 hours (Tables I and II). Further, DDX was found to be almost completely stable to boiling *N* alcoholic alkali for periods up to 4 hours (Table IV). These results suggest that when *p,p'*-DDT is boiled with alcoholic alkali, two reactions are possible, one involving simple dehydrohalogenation to DDX and the other a direct hydrolysis of the $-CCl_3$ group to $-COOH$. In this latter reaction it is immaterial whether two or three chlorine atoms are hydrolysed simultaneously. If three are removed, the acid is produced directly as in the equation above. A bimolecular attack on this group, however, would displace two chlorine atoms to form the acid chloride, which would then yield the acid by secondary hydrolysis.

That DDX yields negligible chloride ion on boiling with *N* alcoholic alkali supports the view that this substance is not an intermediate product in the formation of bis-(*p*-chlorophenyl)-acetic acid from *p,p'*-DDT. There remains, however, the possibility that chloride displaced from *p,p'*-DDT in dehydrohalogenation might influence the stability of the produced DDX to boiling alcoholic alkali. To test this possibility a series of experiments with DDX were made in which potassium chloride was added equivalent in amount to that which would be present if the substance had been formed *in situ* from *p,p'*-DDT. Again, the alcoholic alkali had little action (Table IV). This stability of DDX is not surprising in view of the known resistance to hydrolysis of compounds containing chlorine attached to an unsaturated carbon atom. When more drastic conditions were employed, however, such as boiling DDX with 25 per cent. alcoholic potash for 16 hours under reflux or heating with sodium ethoxide in a sealed tube at 120° C., bis-(*p*-chlorophenyl)-acetic acid was produced.



The dehydrohalogenation experiments carried out at lower temperatures (Table III), showed the conversion of *p,p'*-DDT to DDX to proceed rapidly and quantitatively, and even after three hours the alkali was apparently without further action.* It will be noted that with a concentration of alkali of only 0·1 *N* the reaction was almost complete within 30 minutes (Table III).

It would therefore appear that, although boiling under reflux with alcoholic alkali enables a reaction, undesirable from the analytical point of view, to take place, the action of this reagent on *p,p'*-DDT at laboratory temperatures is mainly one of simple dehydrohalogenation. It is of interest here to note that Cristol and Haller[12] found that *o,p'*-DDT treated with ethanolic sodium hydroxide at room temperatures for periods up to four days yields only one molecule of hydrogen chloride per molecule.

The present results clearly indicate that with *p,p'*-DDT, treatment with 0·1 *N* alcoholic alkali for 30–60 minutes provides more suitable conditions for quantitative dehydrochlorination than those described by Gunther.[2] Experiments are being continued and further results will be communicated in a later paper.

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* That traces of bis(*p*-chlorophenyl)-acetic acid can be produced under these conditions has been indicated by experiments now in progress. The amount produced, however, apparently has an insignificant effect in the estimation.

SUMMARY

When boiled under reflux with *N* alcoholic alkali, *p,p'*-DDT has been shown to lose slightly more than one gram-ion of chloride per mole, indicating that the reaction is not one of simple dehydrohalogenation. This was confirmed by the isolation of bis-(*p*-chlorophenyl) acetic acid as well as DDX from the products of the reaction. This acid could only be produced from DDX by drastic treatment such as prolonged boiling with concentrated alcoholic potash or heating with alcoholic sodium ethoxide in a sealed tube; boiling under reflux with *N* alcoholic alkali for periods up to 4 hours had apparently no effect.

These results indicate that by boiling *p,p'*-DDT with *N* alcoholic alkali the acid can be formed directly by a hydrolysis of $-CCl_3$ to $-COOH$ with consequent liberation of three chloride ions per molecule, and that DDX is not an intermediate product of the reaction. The presence of potassium chloride was found not to affect the stability of DDX to boiling *N*-alcoholic alkali.

The action of *N* and 0·1 *N* alcoholic alkali on *p,p'*-DDT at lower temperatures appeared to be one of simple dehydrohalogenation, and it is suggested that such milder treatment will enable more accurate estimation of this isomer than the procedure recommended by other workers.

Three derivatives of bis-(*p*-chlorophenyl)-acetic acid are described.

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November, 1946

The Estimation of Traces of Mercury in Urine

BY R. F. MILTON AND J. L. HOSKINS

INTRODUCTION—

In the course of an investigation into the effect of mercury upon the health of workers in a thermometer factory and a chemical factory, published elsewhere,[1] it became necessary to make estimations of the minute quantities of mercury excreted in the urine in such circumstances.

From preliminary work on various methods tried for this purpose, only two seemed at all promising. The method due to Stock and Zimmerman[2] was investigated at great length. This consists of oxidation of the urine, electro-deposition of the mercury on a gold electrode, volatilisation from the electrode into a capillary tube, centrifuging of the droplets into a single drop and micro-metric measurement of the diameter of the globule. This technique was found to be so difficult to reproduce, owing to loss of mercury in the glass, film formation, oxidation and so forth, that it was abandoned. The second method involved the use of dithizone and subsequent colorimetric estimation of the mercury complex.

Dithizone undergoes complex formation with a whole range of metals, and its use as an analytical reagent has been admirably expounded by Sandell.[3] Significantly, a group of metals lying close together in the periodic table are capable of forming dithizonates. These are:—

Mn Fe Co Ni Cu Zn
Pd Ag Cd In Sn
Pt Au Hg Ti Pb Bi

Dithizone may form with metals compounds of both keto and enol types, the former containing twice as much metal as the latter.

The enol form generally occurs in alkaline or neutral solution or in absence of an excess of dithizone. Acidification and addition of excess of dithizone usually results in transformation of the metal complex into the keto form. Alternatively, alkalisation may convert it into the enol form.

Dithizone readily undergoes oxidation to diphenylthiocarbodiazone, which is always present in the solid reagent as purchased. The oxidation product dissolves in chloroform or similar solvents to give a yellow-brown solution which interferes with colorimetric estimations. It is however insoluble in dilute ammonia and use is made of this fact to purify the commercial preparation. (The analyst should always purify his reagent—preferably in small quantities—prior to use, by the method advised by Sandell—see below.) The solution of dithizone is unstable to heat and light and for this reason should be freshly prepared and kept in a darkened container in a cool place. Because traces of oxidising agents and certain metals in alkaline solution cause formation of the carbodiazone, hydroxylamine hydrochloride is added to the aqueous solutions of the metals prior to dithizone treatment, to prevent this occurring.

Despite the fact that dithizone reacts to form complexes with so many metals, it may be made almost specific for certain metals by regulating the conditions of reaction. If the acidity of the aqueous solution be adjusted to *p*H 1·0, then dithizone in chloroform will react with copper, mercury, palladium and silver. This behaviour is the basis of the method here proposed, and the assumption is made that the noble metals are completely absent.

APPLICATION OF THE DITHIZONE REAGENT TO THE ESTIMATION OF MERCURY

INTERFERING EFFECT OF COPPER—Copper is always present to an appreciable extent in human urine and biological substances generally and will thus complicate the straightforward estimation of mercury by the direct extraction technique in acid solution (*p*H 1·0).

Various methods have therefore been investigated in an attempt to overcome interference by copper.

1. *Addition of potassium iodide to form a complex with mercury*—In this method acid potassium iodide solution is added and the dithizone extraction is carried out to remove copper, which does not form a complex iodide under these conditions. Subsequent alkalisation breaks up the mercury complex, and re-extraction with dithizone allows removal of the mercury.

Experiments were carried out to ascertain the optimum conditions for this procedure and to ensure that satisfactory recovery of mercury eventually occurs. Fifty μg. of copper were used in each experiment, and extraction was made with 5-ml. quantities of 0·1 per cent. dithizone solution in chloroform.

*p*H of solution	No. of extractions to give complete removal of copper
1·0 to 2·0	10
2·0 to 4·0	5
4·0 to 6·0	3

Thus adequate removal of copper will occur only at about *p*H 5·0. It was then necessary to ascertain whether satisfactory recovery of mercury could be ensured under these conditions, and a similar experiment was carried out in which mixtures of copper and mercury were extracted.

Cu taken μg.	Hg taken μg.	*p*H	Cu extraction	Hg found μg.
50	25	5·0	Complete	7
50	50	5·0	,,	16
50	25	1·0	Incomplete	18
50	50	1·0	,,	43

From these experiments it was concluded that copper is not completely extracted by dithizone in presence of potassium iodide unless the *p*H of the solution is at least 5·0. At this *p*H, however, appreciable quantities of mercury are also extracted.

2. *Formation of rubeanic acid complex with copper*—Copper forms a complex with rubeanic acid. Extraction of copper with dithizone is thus prevented. Experiments with solutions containing copper and mercury (see below) indicate that at *p*H 1·0 incomplete precipitation of copper results, leading to enhanced dithizone extraction. If the *p*H of the

solution is increased dithizone will not completely extract mercury, which appears to be adsorbed on the copper complex.

Cu taken μg.	Hg taken μg.	*p*H	Hg found μg.
50	25	1·0	Copper pptn. incomplete; result masked
,,	50	1·0	,, ,, ,, ,, ,,
,,	25	4·0	21
,,	50	4·0	38
,,	20	7·2	18
,,	50	7·2	38

3. *Removal of mercury as mercuric chloride by extraction with ether*—Mercuric chloride is extracted by ether from acid solutions. Attempts to utilise this property for separation from copper did not meet with complete success. Incomplete recovery of mercury resulted, although three extractions with 20 ml. of ether were carried out from 10 ml. of aqueous solution.

Hg present μg.	Cu present μg.	Hg found μg.	Cu extracted
50	500	46	Nil
50	—	43	—
25	—	23	—
50	—	48	—

4. *Selective extraction of mercury by very dilute dithizone solution*—In strongly acid solution, preferential complex formation with mercury and the noble metals occurs, *if the dithizone is not added in excess.* The sudden change in colour indicates the end of the extraction. This method was tested, with various concentrations of hydrochloric acid in the aqueous phase. Preliminary investigation indicated that a 0·005 per cent. solution of dithizone in chloroform was satisfactory for extraction, and that shaking 25 ml. of aqueous solution with repeated 2-ml. quantities of this chloroform solution provided a satisfactory technique.

Hg present μg.	Cu present μg.	Concn. of HCl	Hg found μg.	Cu effect
50	500	0·05 *N*	60	Contamination of Hg colour
50	500	0·075 *N*	50	nil
50	500	0·10 *N*	50	nil
50	500	0·20 *N*	47	nil
50	500	0·30 *N*	42	nil

These results indicate that small quantities of mercury may be selectively extracted from hydrochloric acid solution in the presence of at least ten times the quantity of copper, provided that the solution is more acid than 0·075 *N*. If, however, the acidity is greater than 0·15 *N* incomplete removal of mercury results.

To confirm the efficiency of the separation and recovery technique, a series of mercury solutions containing 0·5 mg. of copper were subjected to the extraction technique and a graph plotted of colour readings against concentrations, using the Spekker absorptiometer fitted with violet filter No. 8. The technique was as follows:

Solutions containing 0 to 50 μg. of mercury and 0·5 mg. of copper were acidified to 0·1 *N* with concentrated hydrochloric acid solution. The solution was then extracted with successive 1-ml. quantities of a 0·005 per cent. solution of dithizone in chloroform until the characteristic golden yellow colour due to mercury dithizonate was no longer exhibited and green colour due to unchanged dithizone was apparent.

The combined extracts were made up to 15 ml. with the dithizone solution in chloroform and shaken with two 10 ml. quantities of 5 per cent. ammonia to remove excess of dithizone. The mercury dithizonate remained in the chloroform layer, but its acid golden brown colour was now changed by the ammonia treatment to the yellow of the keto form, which is the more stable condition. After the chloroform solution had been shaken with exsiccated sodium sulphate and filtered, the absorption was measured in the Spekker photo-electric absorptiometer. The results are given below; they show this method of procedure to be quite satisfactory.

Mercury present, μg.	0	10	20	30	40	50
Spekker reading	0·07	0·16	0·25	0·34	0·43	0·52
Reading corrected for blank, ×100 ..	—	9	18	27	36	45

APPLICATION OF THE METHOD TO THE ESTIMATION OF MERCURY IN URINE

1. OXIDATION OF URINE—

Great care must be observed during the oxidation of organic matter to avoid loss of mercury by volatilisation. Whatever oxidation procedure is adopted, the operation is best carried out with use of a reflux condenser and in all-glass apparatus. It is advisable to use a 2-litre flask with a twelve-inch water-cooled condenser fitted in the neck, since the rise in boiling-point due to removal of water predisposes to volatilisation losses.

Of the various oxidising procedures tried, that involving the use of sulphuric acid and permanganate was the most rapid and gave the most concordant results.

2. SEPARATION OF MERCURY FROM SOLUTION AS THE SULPHIDE—

Prior to estimation with dithizone, it is essential to separate the mercury from the oxidised solution. This is effected by precipitation as the sulphide, using another metal of the same group as "collector." By this technique, a quantity of an appropriate metal salt is added to the solution. On treatment with hydrogen sulphide the minute amount of mercury sulphide, which would otherwise remain in colloidal suspension, is co-precipitated. Of the various metals chosen for this purpose, arsenic and antimony were most satisfactory as they do not form dithizonates. At the concentration of acid used for dithizonate extraction, antimony tends to be precipitated as the oxychloride and this results in low mercury recoveries. Arsenic, however, exhibits no such complications and proved to be excellent for the purpose. The combined sulphides are separated by centrifuging and dissolved in a mixture of nitric and hydrochloric acids. Nitric acid is then destroyed by addition of hydroxylamine and, after appropriate acidity adjustment, extraction with dithizone solution and estimation of colour follow.

3. RECOMMENDED PROCEDURE—

To about 1 litre of urine (preferably a 24-hour collection) in a 2-litre flask, add 100 ml. of sulphuric acid and 20 g. of potassium permanganate. Heat gently under reflux for about 2 hours to oxidise organic matter and then decolorise excess of permanganate by careful additions of crystals of oxalic acid.

Add 1 ml. of 10 per cent. sodium arsenate solution and pass in hydrogen sulphide for 3 minutes. Then heat the contents of the flask to boiling-point in order to coagulate the precipitate and allow it to settle overnight. Separation of the precipitate is best carried out by centrifuging, first in 250-ml. buckets and then finally concentrating in 15-ml. tubes, but filtration by the filter stick technique may also be used. Wash the precipitate once with water and then dissolve it in 0·4 ml. of nitric and 1 ml. of hydrochloric acids. Warm if necessary to aid solution and to oxidise sulphide to sulphate. Add about 20 ml. of water and 1 g. of hydroxylamine hydrochloride and then make up to 100 ml. with water. With strict adherence to this technique, no further additions of acid should be necessary, but it is well to check that the acidity of the solution is of the order of 0·1 N before proceeding to the extraction stage. Next transfer to a separating funnel and shake with successive 1-ml. portions of a freshly prepared 0·005 per cent. solution of purified* dithizone in chloroform, until the golden brown mercury colour is no longer apparent in the chloroform layer. Unite the chloroform extracts and make them up to 15 ml. with the dithizone solution (or proportions of 15 ml. if more has been added). Remove excess of dithizone by shaking twice with 10 ml. of diluted ammonia (1 in 20). Clarify the chloroform solution by addition of 1 g. of exsiccated sodium sulphate and after filtration read in the Spekker absorptiometer, fitted with violet filter No. 8, and compare with a calibration curve previously prepared from standard solutions of mercury.

This method has been used extensively to investigate the toxic effect of mercury metal on workers in a thermometer factory and on workers in a factory preparing mercury salts.

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* *Purification of dithizone*—Dissolve 0·5 g. of dithizone in 50 ml. of chloroform and filter through sintered glass into a dry receiver. Shake with 50 ml. of diluted ammonia (1 in 100) and separate from the chloroform layer. Repeat the ammonia extraction four times, unite the extracts and shake with a small volume of chloroform. After separating and discarding this chloroform washing, make the ammoniacal solution just acid with pure hydrochloric acid to precipitate the dithizone and extract this with four 15-ml. portions of chloroform. Wash the combined chloroform extracts with two equal quantities of water and evaporate the chloroform off at about 50° C. Place in an amber tube, seal and store at low temperature in the dark. Prior to use, about 10 mg. should be repurified by the process described above and the final chloroform solution diluted to the required concentration.

The full results and conclusions have been published elsewhere.[1] The following table shows an example of the mercury excretion in these cases and also that of some laboratory workers considered to be normal.

URINARY MERCURY EXCRETION FROM WORKERS IN A THERMOMETER FACTORY
(Reproduced from *Brit. Journ. Ind. Med.*)

Operation	Volume per 24 hours ml.	Mercury present μg.	Mercury p.p.m.
A. Calibrating	2200	175	0·080
	1040	278	0·267
	1600	136	0·085
B. "Contracting"	2350	2260	0·961
	1300	500	0·385
	2300	3478	1·500
	1500	866	0·577
	1500	580	0·433
C. "Boiling in"	2249	2280	1·017
	1409	1200	0·857
	880	2040	2·329
	1550	1420	0·916
	1230	1250	1·015
D. Finishing	1330	730	0·561
	1160	1700	1·465
	900	800	0·889
	1200	1440	1·200
	2250	1350	0·600
Workers in factory preparing mercury salts	1000	520	0·520
	1900	750	0·395
	2400	1360	0·566
	1900	520	0·273
	1400	530	0·378
Normal controls (laboratory workers)	2250	23	0·010
	750	12	0·016
	2500	50	0·020
	2600	65	0·025
	1800	9	0·005

SUMMARY

(1) A dithizone extraction technique is described for the estimation of small amounts of mercury in presence of the commonly occurring metals, with special reference to copper.

(2) The method is applied to the measurement of the small amounts of mercury that may be found in urine and involves:—

(*a*) Preliminary oxidation of organic matter;
(*b*) Separation of mercury as the sulphide, with arsenic as "collector";
(*c*) Extraction of mercury as dithizone complex by means of extremely dilute dithizone solution in the presence of 0·1 *N* hydrochloric acid;
(*d*) Colorimetric estimation of the dithizone complex in chloroform with the Spekker absorptiometer.

(3) Results obtained by using this technique on urine from normal persons, workers in a thermometer factor and workers preparing mercury salts are given.

Part of this work was carried out at the Medical Research Council's Industrial Medicine Department, London Hospital.

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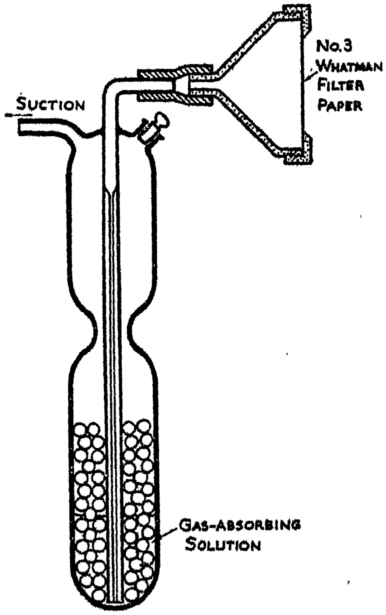
The Estimation of Mercury Compounds in the Atmosphere

BY R. F. MILTON AND W. D. DUFFIELD

THE sampling of mercury in the air involves two distinct problems according to whether the mercury is present as vapour or in particulate form. Mercury vapour has been estimated by a number of methods, all of which have some disadvantages. These are well summarised by Jacobs.[1] A method that involves trapping the mercury vapour in *aqua regia* has been criticised by Norlander[2] on the grounds that the mercuric chloride which forms becomes particulate and passes through solutions as mist. Of the other trapping methods may be mentioned that of Pyankoff,[3] which relies on the action of iodine crystals in the curve of a U-tube to combine with the mercury, that of Polejaeff,[4] in which absorption in iodine and potassium iodide is used, and that of Moldavsky,[5] which utilises gaseous bromine as fixative. All these methods are apt to give incomplete absorption and recovery if the mercury concentrations in the air are low and if the flow rate is raised to practical magnitudes.



In the method used by Stock[6] the air is passed through a tube chilled with either liquid air or carbon-dioxide-snow and ether. The mercury metal so condensed is dissolved in chlorine water and subsequently estimated. With this method we have found great difficulty in disposing of the large volume of ice that separates in the U-tube and have consistently lost some of the mercury in the process.

Filter papers impregnated with selenium sulphide[7] have also been used for estimating mercury vapours, the air being drawn through at a fairly rapid rate. The degree of darkening of the paper indicates the mercury concentration. In this method (*a*) the temperature must be raised to 70° C. for the reaction to occur, and (*b*) it is certain that trapping is incomplete.

Photo-electric methods are available involving measurement of the scattering of the resonance radiation of mercury by mercury vapour.[8] The apparatus, when procurable, is costly.

Mercury salts—These are not accurately estimated by any of the above methods, and therefore when they co-exist with mercury vapour in the atmosphere a specialised technique is necessary. Substances in particulate form in the air, if above a certain particle size, are not trapped in a satisfactory manner by absorption in liquids, as may be readily demonstrated by blowing tobacco smoke into water. Where the particle size is above the limits allowing of absorption by liquids, it is best to use a filter paper through which the air is drawn to absorb the substance. With mercury salts, where the vapour pressure is considerable, absorption on a filter would be accompanied by volatilisation as aspiration proceeded. The loss that this would entail may be overcome by backing the filter with a liquid scrubbing device. This is essential if mercury vapour is also present. The arrangement shown in Fig. 1 is satisfactory for the purpose. The particle filter (a Whatman No. 3) is held in a device such as is used by Fairhall and Sayers[9] for the estimation of lead fumes. This is connected by butted glass tubes to a bead absorption bubbler as described by Milton[10] for the estimation of benzene in the air.

ABSORPTION OF MERCURY VAPOUR—

In order to remove mercury vapour from the air it is first essential to oxidise and fix it as a salt so as to allow of absorption in liquids. We have found alkaline hypobromite to be very satisfactory for this purpose. A solution was prepared by dissolving 4 ml. of bromine in 100 ml. of 8 per cent. sodium hydroxide solution, and 5 ml. of this solution were introduced into the absorption bubbler shown in the figure. Three such bubblers were connected in a series, in front of which was connected a tube containing a little metallic mercury. Air was aspirated over the mercury and through the system at a rate of 2 litres per minute for about 60 minutes, and the mercury-laden atmosphere was thus drawn through the absorption tubes. The contents of each bubbler and the filter were analysed separately for mercury; results are shown in Table I.

TABLE I

Experiment	Hg in 1st bubbler µg.	Hg in 2nd bubbler µg.	Slip from 1st bubbler %
1	408	42	10
2	340	30	9
3	460	37	8
4	125	12	10
5	345	20	6

The slip from the first bubbler is approximately 10 per cent. and this is of the order usually obtained in absorption apparatus of this type. For accurate work it is therefore desirable practice to use two such bubblers in series.

In this absorption system the brownish-yellow hypobromite solution gradually becomes decolorised. This fact does not appear to influence the absorption of the mercury vapour, as is evidenced by Table II. In these experiments three bubblers in series were charged with hypobromite and aspirated with mercury-free air until the solution was decolorised. The apparatus was then connected to a tube containing metallic mercury and air was aspirated through the system. It will be seen that absorption is similar to that with fresh hypobromite solution, as shown in Table I.

TABLE II

Experiment	Hg in 1st bubbler µg.	Hg in 2nd bubbler µg.
1	350	25
2	410	34
3	340	21

ABSORPTION OF MERCURY SALTS—

The particulate nature of mercury salts makes their absorption in liquids an incomplete operation, since particles between 5µ and 25µ may pass through. On the other hand, the use of a filter paper of low resistance in the apparatus mentioned above is very effective for this purpose. In view, however, of the relatively high vapour pressure of the mercury halides, the continual passage of air through the filter results in losses by volatilisation. It is therefore essential to back the particle filter with a liquid absorption device.

ESTIMATION OF MERCURY AFTER ABSORPTION—

(*a*) *Mercury vapour*—Transfer the hypobromite solution containing the mercury to a flask, and rinse out the bubbler three or four times with 5 ml. of water to ensure quantitative transference. Then acidify the solution with a few drops of hydrochloric acid, whereupon the colour turns brown owing to liberation of free bromine. Add, drop by drop, a 50 per cent. aqueous solution of hydroxylamine hydrochloride until the bromine is decolorised. Introduce a small piece of litmus paper into the flask and add dilute sodium hydroxide solution drop by drop until the liquid is just neutral. Then add hydrochloric acid until the acidity of the solution is 0·1 *N*. At this stage the solution (or an aliquot) is ready for estimation of mercury by the dithizone colour method described below.

(*b*) *Mercury in particulate forms*—Transfer the filter paper containing the mercury salts to a small beaker and extract it for about 10 minutes with 2 to 5 ml. of *aqua regia* on a boiling water bath. Pour the acid mass on a sintered filter, or dilute and pour it on to a filter paper, and wash thoroughly, using up to 50 ml. of distilled water for the purpose. To the solution add about 5 ml. of 50 per cent. hydroxylamine hydrochloride solution to destroy nitric compounds and then carefully neutralise the solution, using first 40 per cent. and finally 1 per cent. sodium hydroxide solution. Then add so much hydrochloric acid that the acidity of the solution is eventually 0·1 *N*, and carry out the dithizone colour estimztion as described below.

(*c*) *Colorimetric estimation of mercury*—This is in accordance with the method of Milton and Hoskins previously described.[11] Shake the solution containing mercury in 0·1 *N* hydrochloric acid with successive 1-ml. quantities of a 0·005 per cent. solution of purified dithizone in chloroform until the golden colour due to mercury dithizonate is replaced by the green colour due to excess of reagent. Combine the chloroform extracts, make them up to 15 ml. (or an aliquot) and wash with diluted ammonia (1 in 20) in order to remove excess of dithizone. Then clarify the chloroform extract by shaking it with anhydrous sodium sulphate, filter it, and measure the absorption in a Spekker absorptiometer, using the violet filter No. 8. Refer the result to a graph constructed from readings obtained from standard solutions of mercury submitted to the above extraction technique.

The method was devised in order to carry out estimations of mercury vapour in a thermometer factory and in a factory preparing mercury salts.[12]

A few of these results are reproduced in Table III.

TABLE III

(1) ESTIMATIONS OF MERCURY VAPOUR IN AIR

(Thermometer factory)

	Site	Concentration of mercury µg. per cubic metre
(*a*)	In general shop	25
(*b*)	Where mercury is boiled in stems to remove air	100 108
(*c*)	Where mercury is centrifuged into glass stems	175 70 670 220

Multiple results represent findings on different days

(2) ESTIMATION OF MERCURY SALTS AND MERCURY VAPOUR IN AIR

(Factory manufacturing mercury salts)

	Site	Concentration of Hg µg. per cubic metre	
(*a*)	Weighing booth (Hg metal)	"Vapour"	185
		"Particulate"	nil
(*b*)	Preparation of mercury oxide and mercury iodide	"Vapour"	150
		"Particulate"	130
(*c*)	In sieving room	"Vapour"	160
		"Particulate"	18,000
(*d*)	In sublimate manufacturing room	"Vapour"	125
		"Particulate"	230
(*e*)	In sublimate packing room	"Vapour"	125
		"Particulate"	540

SUMMARY

(1) Methods for the estimation of mercury in the air have been discussed.

(2) A new method using alkaline hypobromite for the trapping of mercury vapour is described.

(3) The collection of particulate mercury in air is also described.

(4) The subsequent estimation of mercury in the samples is outlined.

(5) The method has been applied to the estimation of mercury vapour and mercury salts in factory air, and some results are tabulated.

Part of this work was carried out at the Medical Research Council's Industrial Medicine Department, London Hospital.

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The Precipitation of Aluminium and its Separation from Cobalt, Chromium, Nickel or Zinc by means of Ammonium Benzoate

By A. A. SMALES

INTRODUCTION—

The analysis of materials containing aluminium together with cobalt, chromium, nickel or zinc is often required. The classical method of separation of these metals from aluminium is to remove them from a sulphuric or perchloric acid solution by electrolysis with a mercury cathode. This procedure is time-consuming, however, and a direct precipitation of the aluminium would be preferable. Precipitation with ammonium hydroxide (in the case of chromium after oxidation of Cr^{III} to Cr^{VI}) is ruled out because occlusion by aluminium hydroxide makes repeated precipitations necessary. Several other direct precipitants have been suggested, but of these only ammonium benzoate[1,2,3] appeared to be suitable for our purpose.

This ammonium benzoate precipitation has been attempted at Billingham on one or two occasions previously and, although some good results have been obtained, a few were poor, and hence no confidence could be placed in the procedure used, which was that of Kolthoff.[1] Thus it was first necessary to standardise conditions of precipitation.

The use of benzoate for separation of aluminium from chromium does not appear to have been mentioned previously and it was therefore necessary to determine the efficiency of the separation. It was considered also that a more direct method of determining the cobalt, nickel and zinc in the final alumina would be preferable to Kolthoff's indirect method.

EXPERIMENTAL

(1) PRECIPITATION CONDITIONS—

Instructions given by Kolthoff ("cautiously neutralise with dilute ammonia solution until the precipitate redissolves very slowly, then add 1 ml. of glacial acetic acid and 1 g. of ammonium chloride"), allow a fairly wide variation in the *p*H at which precipitation takes place. Therefore the first step was to find the optimum *p*H for precipitation.

A standard aluminium solution containing approximately 1 mg. of aluminium per ml. was prepared from ammonium alum and standardised gravimetrically by precipitation with ammonium hydroxide; 50 ml. gave 93·0 mg. of alumina.

Several 50-ml. lots were measured out and diluted to 100 ml., and to each was added a solution containing 1 g. of ammonium chloride and 2 g. of ammonium benzoate. The *p*H of each was adjusted by addition of *N* hydrochloric acid or ammonia in the cold, to give values of 2·5, 3, 3·5, 4, 5, 5·5, 6 and 7, respectively. (The *p*H was measured by means of a Marconi-Ekco *p*H meter with glass electrode.) The solutions were then boiled, the precipitates were allowed to settle for 30 minutes, filtered through 12·5-cm. Whatman No. 40 papers and washed with a warm solution containing 10 g. of ammonium benzoate and 20 ml. of glacial acetic acid per litre. They were ignited to constant weight, first in a muffle furnace, and finally over a blast lamp. The weights of alumina so obtained are shown in Table I.

TABLE I

EFFECT OF VARIATION OF *p*H ON THE PRECIPITATION OF ALUMINIUM BENZOATE

*p*H value ..	2·5	3	3·5	4	5	5·5	6	7
Al_2O_3, mg. ..	70·9	85·2	93·2	93·0	93·0	93·0	93·5	93·3

The appearance of the precipitates at the various *p*H values was informative. Those at *p*H 2·5, 3 and 3·5 gave trouble owing to crystallisation of benzoic acid during filtration. Those at *p*H 4 and 5 were granular and filtered easily, whilst those at *p*H 5·5, 6 and 7 became gelatinous and difficult to filter—apparently the hydroxide, and not the benzoate, began to form the major part of the precipitate at these higher *p*H values. Thus, the optimum *p*H range is from 3·5 to 5, preferably 4, as occlusion of other ions might be expected to be least at the lower *p*H.

For routine work an easier method of *p*H control than the use of a glass electrode *p*H meter was desirable. Hence bromophenol blue (*p*H 3–3·6) was used as indicator in subsequent work, although for information the *p*H value of all filtrates was also measured by the glass electrode *p*H meter.

The next step was to carry out benzoate precipitations on pure aluminium solutions, with bromophenol blue as indicator, to confirm that (*a*) quantitative results, and (*b*) adequate *p*H control, were obtainable. Table II shows results of experiments in which 25- and 50-ml. lots of the standard aluminium solution were treated with 1 g. of ammonium chloride and 2 g. of ammonium benzoate, acidified, heated and then treated with dilute ammonia from a burette until just blue to bromophenol blue. The solutions were boiled and filtered and the precipitates ignited as before.

TABLE II

*p*H CONTROL AND ALUMINIUM RECOVERY WHEN BROMOPHENOL BLUE INDICATOR WAS USED

Std. Al solution, ml.	Al_2O_3 recovered, mg.	*p*H of filtrate
25	46·4	5·6
25	46·5	4·8
50	92·6	6·4
50	93·0	5·1

Thus although quantitative results were obtained *p*H control (and presumably therefore occlusion control) was poor.

It was therefore necessary to have more buffering capacity in the *p*H 4 range; so in the following precipitations 2 g. of ammonium acetate were added in addition to the 1 g. of ammonium chloride and 2 g. of ammonium benzoate. The conditions of precipitation were also standardised to give consistently a granular, rapidly filterable precipitate. The conditions under which this occurs result from the following procedure:—To the slightly acid (usually hydrochloric acid) solution containing aluminium, of volume 250–300 ml., add 1 g. of ammonium chloride, 20 ml. of 10 per cent. ammonium acetate solution, 20 ml. of 10 per cent. ammonium benzoate solution and 2 ml. of bromophenol blue indicator solution. Heat to 80° C., add sufficient hydrochloric acid to dissolve any precipitated aluminium benzoate (the benzoic acid, which precipitates in the cold, is soluble at 80° C.) and add dilute ammonia from a burette slowly, and with shaking, until the indicator just begins to change colour and precipitation *just* begins. At this point, boil the solution for one or two minutes, whereupon further precipitation takes place giving a granular precipitate. Usually at this point the solution becomes just acid again. Continue the addition of dilute ammonia from the burette until the indicator just gives the red-blue colour characteristic of *p*H 3·5 to 4. Boil the solution gently for 2–3 minutes and place on a water bath for 30 minutes to allow the precipitate to settle. Filter through a Whatman No. 40 paper and wash 10 times with hot wash liquor containing 10 g. of ammonium benzoate and 20 ml. of glacial acetic acid per litre (*p*H 3·8).

Results of precipitations carried out under these conditions are shown in Table III, 50 ml. of the standard aluminium solution being used.

TABLE III

*p*H CONTROL AND ALUMINIUM RECOVERY UNDER NEW CONDITIONS OF PRECIPITATION

Al_2O_3 recovered, mg...	93·5	93·0	92·8	93·0
*p*H of filtrate ..	3·6	3·8	4·05	4·0

Thus, *p*H control now appeared satisfactory. Results of experiments involving separations, described below, give further evidence of this.

2. SEPARATIONS—

The efficiency of separations was measured (*a*) by weighing the alumina after ignition of the benzoate precipitate to constant weight and (*b*) by polarographic determinations (see Note 1) of the "contaminating" metal after fusion of the "contaminated" alumina with 2 g. of potassium hydrogen sulphate. The fusions containing chromium, nickel or zinc were dissolved in water, diluted to 100 ml. and polarographed over a suitable voltage range. (Two or three drops of sulphurous acid were added to the chromium solution and excess was boiled off, to make quite certain that chromium was present as $Cr^{\cdots}$). The fusions containing cobalt were dissolved in water, 1 g. of tartaric acid was added and the solution made ammoniacal before dilution to 100 ml. In all four cases standard polarograms were obtained for known amounts of the metals under similar conditions. That is, for chromium, nickel or zinc, four lots of 2 g. of potassium hydrogen sulphate and 50 mg. of aluminium as alumina were fused, and to the dissolved fusions were added 0, 0·5, 1 and 2 mg. of the metal in question (as the sulphate) before diluting; for cobalt the dissolved fusions were made ammoniacal after addition of 1 g. of tartaric acid and 0, 0·5, 1 and 2 mg. of cobalt (also as sulphate) and then diluted to 100 ml.

In each experiment two lots of 50 ml. (≡ 93·0 mg. of alumina) of the standard aluminium solution were taken, together with 50 and 100 mg. respectively of the metal in question, chromium as potassium dichromate (see Note 2), and cobalt, nickel and zinc as sulphates; precipitation was carried out as described above. Table IV gives the results obtained.

TABLE IV

SEPARATION OF ALUMINIUM FROM COBALT, CHROMIUM$^{\mathrm{VI}}$, NICKEL OR ZINC BY MEANS OF A SINGLE AMMONIUM BENZOATE PRECIPITATION

Alien metal, mg.	Wt. of oxide obtained, mg. (should be 93·0 for perfect separation)	Wt. of alien metal (not oxide) determined polarographically, mg.	*p*H of filtrate
50 Co	92·8	0·1	3·65
100 ,,	93·4	0·1	3·7
50 Cr$^{\mathrm{VI}}$	93·2	0·1	3·8
100 ,,	93·4	0·1	3·8
50 Ni	93·4	0·5	3·8
100 ,,	93·7	0·5	3·9
50 Zn	93·4	0·3	3·8
100 ,,	94·0	0·7	3·8

From these results it can be seen that, for the quantities given, separation by a single precipitation is satisfactory at least for cobalt$^{\mathrm{II}}$ and chromium$^{\mathrm{VI}}$.

A further series of experiments on 50-ml. portions of the standard aluminium solution, with 100 mg. of cobalt, chromium$^{\mathrm{VI}}$, nickel or zinc, was carried out with a reprecipitation, *i.e.*, the original benzoate precipitate, after being washed, was dissolved off the paper by hydrochloric acid and the aluminium precipitated a second time, again at a volume of 250–300 ml., under the same conditions as before. The ignited precipitates were not weighed, however, but simply fused with potassium hydrogen sulphate and polarographed as before. In each case the amount of alien metal in the ignited oxide was less than 0·1 mg.

Thus a double precipitation is entirely satisfactory for the quantities tried, in all four cases.

NOTES—

1. In the polarograph determinations no attempt was made to establish optimum conditions for the accurate determination of each metal concerned. The polarograph was the most convenient means of quickly determining the amount of alien metal present in the residual alumina and the most convenient supporting electrolyte happened to be a potassium hydrogen sulphate solution, which gave waves sufficiently good for the purpose and which were easily reproducible with chromium, nickel or zinc. This medium was not suitable for cobalt however, but neutralisation of the potassium hydrogen sulphate solution by ammonia in the presence of tartaric acid gave suitable conditions for a good reduction wave for cobalt.

In each case a saturated calomel electrode was used as anode and waves were obtained at a sensitivity of 1/10. The polarograph used was a Cambridge instrument, and solutions were de-oxygenated by means of a stream of electrolytic hydrogen before the polarograms were taken.

2. The character of the precipitate of aluminium benzoate seems to be particularly important in the separation from chromium$^{\mathrm{VI}}$, *e.g.*, an experiment in which 50 mg. of aluminium were precipitated in presence of 50 and 100 mg. of chromium$^{\mathrm{VI}}$ and in which the aluminium benzoate was purposely precipitated as a very flocculent mass by rapid addition of ammonia, gave final alumina containing 1·5 and 2 mg. of chromium respectively.

SUMMARY

Optimum conditions have been fixed for precipitation of aluminium as benzoate. It has been shown that a single precipitation will give a practicable separation of 50 mg. of aluminium from quantities of cobalt$^{\mathrm{II}}$ and chromium$^{\mathrm{VI}}$ up to at least 100 mg., but that a double precipitation is desirable for similar amounts of nickel$^{\mathrm{II}}$ or zinc.

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The Determination of Copper and Arsenic in Iron Pyrites by the Method of Internal Electrolysis

BY W. CULE DAVIES AND CYRIL KEY

THE method commonly used for the determination of copper in iron pyrites[1] involves attacking the ore with acid, precipitation of copper sulphide and final deposition of the copper electrolytically. The method is long, tedious and liable to considerable manipulative error.

We have found that the method of internal electrolysis developed by Sand[2] and applied to the determination of copper in steel by Fife and Torrance[3] can be so modified that the determination of copper in a pyrites is simple, direct, rapid and capable of high accuracy.

Some samples of pyrites, the copper content of which had been determined by other methods and other analysts, were examined by the method of internal electrolysis (Table I) with a platinum gauze cathode and the apparatus described by Davies and Key.[4] Mixtures of concentrated nitric and hydrochloric acids or of nitric and sulphuric acids were used for breaking down the ore. Hydrazine sulphate or hydroxylamine hydrochloride was used to reduce the ferric salt, the much more soluble hydroxylamine salt being preferred because of its superior reducing properties in these solutions. It was found difficult to reduce all the ferric iron in the catholyte by this means, but the residual amount was soon reduced in the electrolysis. Some other reducing agents, *e.g.*, zinc and acid and sodium hypophosphite, precipitate copper; sulphur dioxide is not so satisfactory as the hydrazine or hydroxylamine salt, since when it is used the copper tends to be loosely held on the cathode.

METHODS

METHOD A. DETERMINATION OF COPPER IN ABSENCE OF ARSENIC—

Treat pyrites (3 g., or sufficient to give up to 50 mg. of copper deposit), which has been ground to pass a 100-mesh sieve and dried at 105° C., in a covered dish with 25 to 30 ml. of an acid mixture (70 vols. of concentrated nitric acid with 30 vols. of concentrated sulphuric acid) added in separate small portions. Finally, heat the mixture until copious fuming occurs. Wash the cooled product into a beaker with water and reheat. Remove insoluble matter by filtration, and treat filtrate and washings (about 300 ml.) with ammonia until a slight permanent precipitate appears. Then to the almost boiling solution gradually add 2 to 3 g. of hydroxylamine hydrochloride. Finally, electrolyse at 70° C. for 50 to 75 minutes. Iron rods are used as anodes, with an anolyte of acidified ferrous sulphate solution (containing 5 g. of iron and 3 ml. of concentrated sulphuric acid per 100 ml.) contained in small parchment bags. Some results are given in column 2 of Table I.

TABLE I

DETERMINATION OF COPPER IN PYRITES

	Copper found, %				
			By other analysts using other methods		
Pyrites sample No.	By Method A	By Method B	(1)	(2)	(3)
1	1·73 1·71 1·71	1·72 1·72	1·88	1·71	1·78
2	1·63 1·62	1·70 1·72	1·70	—	—
3	1·54 1·51 1·55 1·52	1·58 1·58	1·50	1·61	1·55
4	1·85 1·83	1·84 1·86	—	1·90	1·80
5	—	2·28	—	2·35	2·28
6	—	1·76	—	1·71	1·64
7 (contains 0·5% As)	—	1·765	1·76	—	—
8 (copper pyrites)	—	26·2	26·1	—	—

METHOD B. DETERMINATION OF COPPER IN PRESENCE OF ARSENIC—

Dissolve the pyrites, neutralise with ammonia, and reduce with hydroxylamine hydrochloride, as in Method A. Then add a 50 per cent. solution of sodium citrate (40 ml. for about 5 g. of pyrites) and dilute the whole to about 300 ml. If some cuprous oxide is precipitated add enough hydrochloric acid to the hot solution to dissolve this precipitate. This final solution is the catholyte. Prepare the anolyte by adding 20 ml. of the sodium citrate solution to 50 ml. of a ferrous sulphate solution containing the equivalent of 5 g. of iron and 3 ml. of concentrated sulphuric acid per 100 ml. Electrolyse as before at 70° C. The time required for complete deposition of 50 mg. of copper is 50 minutes.

Table II gives some results obtained with known mixtures of copper, ferric and arsenic salts. Some results for pyrites by Method B are also given in Table I.

TABLE II

DETERMINATION OF COPPER IN MIXTURES OF COPPER, FERRIC AND ARSENIC SALTS

Composition of mixture			
As (added as sodium arsenite) g.	Fe (added as ferric alum) g.	Cu (added as copper sulphate) g.	Cu found g.
0·0500	2·0	0·0250	0·0250
0·1000	3·0	0·0250	0·0249
0·5000	2·5	0·0250	0·0249
0·0250	4·0	0·0250	0·0250
0·1000	4·0	0·0250	0·0250
0·1000	2·0	0·0500	0·0499
0·1000	4·0	0·0500	0·0500
0·5000	3·0	0·0500	0·0499
0·5000	3·0	0·0300	0·0300

TABLE III

(a) DETERMINATION OF COPPER AND ARSENIC IN SOLUTIONS CONTAINING COPPER SULPHATE, SODIUM ARSENITE AND FERROUS SULPHATE; NO CHLORIDE ION PRESENT

Original quantity of copper g.	Arsenic present g.	Copper added during the electrolysis g.	Total copper and arsenic present g.	Total copper and arsenic found g.
0·010	0·025	0·010	0·045	0·0448
0·010	0·025	0·020	0·055	0·0551
0·015	0·035	0·025	0·075	0·0749
0·010	0·050	0·010	0·070	0·0698
0·025	0·075	0·020	0·120	0·1202
0·005	0·020	0·010	0·035	0·0352

(b) DETERMINATION OF COPPER AND ARSENIC IN SOLUTIONS CONTAINING COPPER, QUINQUEVALENT ARSENIC AND FERROUS IRON

Original quantity of copper g.	Copper found by Method B g.	Arsenic present g.	Arsenic found g.	Iron present as Fe g.	Extra copper added g.
0·0250	0·0250	0·0093	0·0092	2·0	0·0200
0·0440	0·0440	0·0220	0·0215	3·0	0·0200
0·0350	0·0349	0·0080	0·0080	2·5	0·0100
0·0500	0·0500	0·0125	0·0124	2·5	0·0100
0·0330	0·0330	0·0110	0·0109	2·5	0·0100
0·0780	0·0782	0·0310	0·0313	2·0	0·0200
0·0590	0·0585	0·0950	0·0952	2·0	0·0100
0·0	—	0·00485	0·0048	2·0	0·0200

(*c*) DETERMINATION OF COPPER AND ARSENIC IN IRON PYRITES, ETC.

Sample	Copper found by Method B %	Arsenic found by Method C %	Arsenic found by Evans's method[5] %
1	0·496	0·13	0·12
2	0·350	0·12	0·115
3	0·705	0·0875	0·0875
4	1·765	0·525	0·53
5 (pyrites flue dust)	0·01	0·72	0·716
6 (copper-arsenic alloy)	55·6 (55·5 volumetric)	36·2 (36·5 volumetric)	—
7 (sulphuric acid)	—	140 parts per 10^6	132 parts per 10^6
8 Ditto	—	58 Ditto	60 parts per 10^6

METHOD C. DETERMINATION OF ARSENIC IN PYRITES—

Make up the pyrites solution to a convenient volume, and take aliquot parts for copper and arsenic determinations. When determining both elements together, add ammonia in the cold until a faint permanent precipitate appears and evaporate the mixture to about 75 ml. Reduce ferric iron with hydroxylamine hydrochloride, as described in Method A, add 15 ml. of concentrated hydrochloric acid and 5 g. of sodium hypophosphite, and boil the mixture gently under reflux for 20 minutes. Dilute the solution, transfer to the electrolytic cell, make up to about 300 ml. and electrolyse at 70° C. Add 10 ml. of standard copper solution (1 ml. ≡ 1 mg. Cu) to the catholyte after electrolysis has proceeded for 30 minutes. If necessary, make a further addition of standard copper solution 15 minutes later to complete the deposition of arsenic. The anolyte is a ferrous sulphate solution containing the equivalent of 5 g. of iron and 3 ml. of concentrated sulphuric acid per 100 ml. When deposition is complete, remove the platinum gauze cathode, wash successively with water, alcohol, and ether, dry at 100° C. and weigh. Deduction of the extra copper added gives the total amount of copper and arsenic originally present; the amount of arsenic is obtained by deducting the copper as determined by Method B.

ARSENIC IN OTHER MATERIALS—

To determine arsenic in a solution containing tervalent arsenic and copper, add ferrous sulphate solution equivalent to about 2·5 g. of iron, dilute the mixture to 300 ml. and electrolyse at 70° C.

To determine arsenic in other materials containing little or no iron, reduce quinquevalent arsenic with sulphurous acid and remove excess of sulphur dioxide by boiling. After the addition of standard copper and ferrous sulphate solutions, electrolyse the mixture in the usual manner. Alternatively, reduce the arsenic with sodium hypophosphite.

To determine arsenic in sulphuric acid, neutralise the acid with ammonia, reduce arsenic with sodium hypophosphite in 15 to 20 per cent. hydrochloric acid solution, add standard copper and ferrous sulphate solutions, and electrolyse in the usual way.

Treat flue dusts, from pyrites burners, with nitric acid, and remove excess of nitric acid by adding sulphuric acid and evaporating to fuming. Then proceed as for pyrites.

The Whatman diffusion shells used in the determinations to contain the anolyte are slowly attacked by solutions containing hypophosphite and require renewal after one day's continuous use. Where occasional determinations are made, the shells can be preserved for some time, if after being washed they are kept in dilute sulphuric acid.

INFLUENCE OF OTHER ELEMENTS—

Lead and zinc frequently occur in pyrites. Lead is removed as the insoluble sulphate by the nitric - sulphuric acid mixture used for attacking the ore, but it has no adverse influence, even if present, in the citrate solution of Method B. Zinc, being less noble than iron, is not deposited. Cobalt and nickel sometimes occur in traces in pyrites, but would not be deposited. Silver, which sometimes occurs in traces, could be removed as chloride. Antimony would not normally be removed and if in the tervalent state would be deposited in the acid solution of Method A, but not in the citrate solution of Method B; its occurrence, however, in pyrites is rare, and even then in very small amount. Tin, bismuth and mercury, which might cause difficulties, seldom occur in pyrites: cadmium does not interfere. Table IV gives the results of some determinations of copper in presence of certain other elements.

TABLE IV

DETERMINATION OF COPPER IN PRESENCE OF OTHER ELEMENTS

Copper present g.	Copper found g.	Method	Other metals present
0·0250	0·0251	A	Co, Ni—0·5 g. of each
0·0250	0·0250	A	Cd—0·1 g.
0·0250	0·0250	B	Co, Ni, As—0·5 g. of each
0·0400	0·0399	B	Ditto
0·0250	0·0251	B	Sn—1 g.; As—0·5 g.
0·0500	0·0499	B	Ditto
0·0400	0·0400	B	Cd—0·1 g.
0·0250	0·0250	B	Pb—0·075 g.
0·0400	0·0400	B	Sb—0·5 g.

DISCUSSION

The internal electrolysis method described by Fife and Torrance[5] for the determination of copper in steel, as would be expected, can be applied to the determination of copper in iron pyrites (Method A). In determinations by Method A with certain pyrites, however, the copper deposit on the cathode darkened slightly towards the end of the electrolysis. This was due to simultaneous deposition of arsenic. Provided the arsenic content of the pyrites is not more than 100 parts per million, the error introduced is negligible, for in any event the arsenic is incompletely deposited under these conditions. Many pyrites ores contain appreciable amounts of arsenic, and, therefore, a method of determining copper in them was sought, not involving removal of arsenic by chemical separation. It was established that by carrying out the electrolysis in an almost neutral solution to which excess of sodium citrate had been added, copper was deposited completely and rapidly, without simultaneous deposition of arsenic. Method B, for the determination of copper in presence of arsenic, makes use of this discovery.

Since arsenic is deposited with copper on the cathode from a suitable solution, it appeared possible that both elements could be determined together. Solutions containing known amounts of arsenic (as arsenite), ferrous sulphate and copper were electrolysed under the conditions of Method A. The cathode deposit weighed about 1·5 mg. less than theoretical, but contained all the copper originally present. The electrolysis was repeated, but this time a further quantity of a standard copper solution was added after the electrolysis had proceeded for some time. A layer of copper containing the remainder of the arsenic was deposited over the first, dark-coloured, combined deposit of copper and arsenic. The total weight of copper, deducted from the final weight of the combined deposit, gave the weight of arsenic, and this was now equal to the whole quantity originally present. It was found to be important to add a sufficient excess of standard copper solution during the electrolysis to ensure complete deposition of arsenic. The time required for the deposition of 10 mg. of arsenic, using a total of 50 mg. of copper, was about 55 minutes at 70° C., the additional 10 mg. of copper being added after the electrolysis had proceeded for 30 minutes. If the arsenic was originally present in the quinquevalent state it was necessary first to reduce it to the tervalent state. It was found that sodium hypophosphite in hydrochloric acid was the most satisfactory reducing agent. The solution to be reduced should contain hydrochloric acid equivalent to 15 to 25 ml. of the concentrated acid per 100 ml. of solution.

According to Torrance,[6] quantitative electro-deposition of arsenic from a solutionc ontaining it in the tervalent state requires the presence of chloride ion and simultaneous deposition of copper. We now find, however, that, under the conditions we describe for the internal electrolysis method, arsenic is completely deposited with copper even in absence of chloride ion. It should, however, be pointed out that whereas Torrance worked in hydrochloric acid solution of about normal acid concentration, the solutions used in the present methods are almost neutral, so that arsenious acid anions may be present. Such anions may be a possible source of the differences now observed. Table III (*a*) gives a selection of determinations carried out in absence of chloride ion. Table III (*b*) gives some determinations in which chloride ion is present, quinquevalent arsenic being reduced by sodium hypophosphite in hydrochloric acid.

It is not possible completely to deposit the arsenic of a pyrites containing carbonaceous matter, although the copper is satisfactorily deposited. Attempts to oxidise the carbonaceous material were not altogether successful, and many of the oxidising agents tried introduced difficulties in the subsequent electrolysis.

SUMMARY

The internal electrolysis method has been applied to the determination of copper in iron pyrites. For pyrites ore containing appreciable amounts of arsenic, the method is modified to prevent simultaneous deposition of arsenic with copper. It was found that arsenic was not deposited with copper if the electrolysis was conducted in almost neutral solution containing sodium citrate. Details are given for determining arsenic together with copper, by continued electrolysis following further additions of standard copper solution. The influence of possible interfering elements has been studied.

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CENTRAL RESEARCH DEPARTMENT
BROTHERTON & CO., LTD., LEEDS

September, 1946

Notes

A NOTE ON ALCOHOL IN BLOOD AND URINE

DURING the past ten years this Laboratory has often been called upon to determine the alcohol in samples of blood and urine and we have used the Widmark method. From the first it was noticed that the urine figures were invariably higher than those for the blood and that the discrepancy was more pronounced if the samples had been kept overnight before the analysis. In a case where death occurred one morning, a post-mortem examination was carried out in the afternoon, and the blood and urine were analysed for alcohol the following morning. The urine was found to contain 0·37 per cent. of alcohol, whereas the blood contained only 0·22 per cent.

Experiments were carried out and it was found that the concentration of alcohol in blood decreased rapidly on keeping, especially if the samples were not stored in the refrigerator. In one typical case, in 24 hours, even though the sample was stored in the refrigerator, the concentration dropped from 0·09 per cent. to 0·06 per cent.

On the other hand, refrigerator-stored urines were constant for long periods. Three urines, with alcohol contents of 0·14, 0·30 and 0·24 per cent., had the same values after one month's keeping, and one of 0·34 per cent. was unchanged after two months' storage.

It is now the practice of the Laboratory to regard the urine figures as the more reliable, especially if any time elapses between the taking of the sample and the analysis.

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September, 1946

THE DISTRIBUTION OF BARBITURATES IN THE HUMAN BODY

DURING the past twenty years we have examined many exhibits for barbiturates, mostly in cases of suicide. During 1936 in a case (A) where sodium barbitone (medinal) equivalent to 225 grains of barbitone (veronal) had been taken by the deceased a wide range of organs was submitted for examination. The amounts found in the various organs were approximately the same, and the muscle tissue contained a similar amount. It could therefore be concluded that the barbitone was uniformly distributed throughout the body. The results from cases B, C, D and E confirm this.

It will be seen that there is no concentration in the liver or in the brain, although in "*Toxicology*" by McNally, p. 721, there is a reference to a fatal case of poisoning by phenobarbitone (Wright, *Arch. Int. Med.*, 1929, 43, 85) in which the concentration of the drug is given as 90 mg. per 100 grams in the brain, but as only 3 and 5 milligrams in the stomach and liver respectively. Our figures show that there is no concentration in the brain and we are of the opinion that the figures given by McNally are erroneous.

The figures obtained by us indicate that the approximate amount of barbiturate in the body can be calculated from the proportion found in any one organ.

The following table gives the results obtained from five typical cases:

CONCENTRATION, MILLIGRAMS PER 100 GRAMS

Case	Barbiturate found	Liver	Brain	Kidney	Stomach	Stomach contents	Lung	Intestine	Thigh muscle	Urine	Cerebrospinal fluid
A.	Barbitone ..	13	15	14	12	—	12	14	11	37	—
B.	Phenobarbitone	6	6	—	—	—	—	—	—	—	—
C.	Barbitone ..	9	13	9	8	8	—	—	—	—	—
D.	„ ..	20	20	24	—	277	—	—	21	—	—
E.	„ ..	28	—	28	29	—	—	—	—	27	24

In Case D the high concentration in the stomach contents was due to the presence of a large quantity of undissolved drug, death having occurred fairly rapidly after the taking of the drug. The white lumps could be easily picked out and washed and identified.

It will be seen that the figure for urine in Case E corresponds to that in the organs examined but in Case A the figure for urine is much higher than the figures for the organs. We can offer no explanation for this difference.

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ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

South African Fish Products. Part XXII. Periodic Acid as Reagent in the Analysis of the Unsaponifiable Fraction of Natural Fats. M. L. Karnovsky and W. S. Rapson (*J. Soc. Chem. Ind.*, 1946, **65**, 138–140)—Since the α-glyceryl ethers are the only 1 : 2-glycols recorded as components of unsaponifiable fractions of natural fats, their estimation by means of such reagents as lead tetra-acetate or periodic acid, which act specifically on 1 : 2-glycols, has been explored. Both these reagents effect the oxidation of α-glyceryl ethers according to the scheme

$$CH_2OR.CHOH.CH_2OH \rightarrow CH_2OR.CHO + CH_2O$$

and the course of the reaction has been followed by estimation both of the reagent consumed and of the formaldehyde generated. The use of lead tetra-acetate was abandoned because it attacked other constituents of the unsaponifiable matter, especially squalene. Periodic acid, applied first in acetic acid and later in ethyl alcohol and ethyl acetate solutions, proved to be a more selective reagent. It was considered desirable that the method of estimating α-glyceryl ethers should be a direct sequel to the ordinary S.P.A. method for determining unsaponifiable matter, using 2 g. of fat (or more when necessary), and should be applicable to the very small amounts of material often obtained in that method. Consequently, conditions were determined for the use of 0·0325 N periodic acid, and, under these conditions, the oxidation of batyl alcohol was found to be complete in 4 hr. Acetic acid was not used in the final procedure owing to the difficulty of adjusting the pH of the reaction mixture in its presence in the subsequent method for estimating the excess of oxidising agent with sodium arsenite. Under the conditions finally selected, the sample of batyl alcohol available assayed at 98·13 per cent. pure on the basis of reagent consumed, and at 99·9 per cent. on the basis of formaldehyde generated in the reaction. Samples of unsaponifiable matter were prepared by dissolving them in either ethyl alcohol or ethyl acetate, or in mixtures of both, there being no interference by either of these solvents.

Procedure—To prepare the periodic acid reagent, dissolve 2·4 g. of sodium paraperiodate in 15 ml. of water containing 0·8 ml. of sulphuric acid, and add 235 ml. of 96 per cent. alcohol, purified, if necessary, by distillation from potassium hydroxide in presence of aluminium turnings. Remove the white precipitate by filtration, and allow the reagent to stand overnight. The standard sodium arsenite solution is about 0·02 N, and contains 10 g. of sodium bicarbonate per litre, and is adjusted to give a back-titration figure of 3 to 5 ml. of 0·02 N iodine for 5 ml. of periodic acid in a blank determination. It is useful to know, in each batch of solutions, the direct titre of the standard iodine solution used (0·02 N) against the sodium arsenite solution so that, in any experiment, it is immediately evident that the necessary excess of periodic acid has been added.

Isolate the unsaponifiable matter by the method of the S.P.A. (ANALYST, 1933, 58, 203), but with four ether extractions instead of three. To 5 ml. of a solution of 35 to 50 mg. of unsaponifiable matter in 5 ml. of alcohol or ethyl acetate in a conical flask, add 5 ml. of periodic acid reagent, and swirl the mixture at 10-min. intervals for the first half-hour and, subsequently, at half-hourly intervals. If the α-glyceryl content is high, a white precipitate of iodic acid separates within 10 min. At the end of four hours, add 25 ml. of saturated sodium bicarbonate solution, measured from a cylinder, and 25 ml. of the standard sodium arsenite solution from a pipette. Organic matter will be precipitated (this is less evident when ethyl acetate is the solvent) and the iodic acid will dissolve. Add 3 ml. of 10 per cent. potassium iodide solution, swirl the mixture thoroughly, allow it to stand for 15 min., rinse down the sides of the flask with about 15 ml. of water, and titrate the excess of sodium arsenite with 0·02 N iodine, starch being used as indicator. Carry out blank determinations simultaneously, 5 ml. of the solvent instead of the sample solution being used. From the result of the titration, calculate the percentage of α-glyceryl ether as selachyl alcohol (50 mg. of selachyl alcohol ≡ 0·146 mg.-mol.).

To determine the formaldehyde generated in the reaction, add to the oxidation mixture that has been allowed to stand for 4 hr. (*supra*) 30 ml. of water, 9 ml. of N hydrochloric acid, and 3 ml. of sodium arsenite. Mix thoroughly, and when the colour formed has disappeared, add 60 ml. of a buffer solution made by mixing 1 volume of 1·7 N hydrochloric acid with 2 volumes of 1·7 N sodium acetate, and filter through a pad of filter-aid, gentle suction being used. Wash the flask and pad with another 20 ml. of buffer solution, and then with about 10 ml. of water from a wash-bottle. To the clear filtrate (*p*H 4·6), add 2 ml. of 5 per cent. alcoholic dimedone solution, shake the mixture thoroughly, and allow it to stand for 18 hr. Collect the precipitate in a tared, sintered glass crucible, wash it with distilled water, dry the crucible at 85° C. for 20 min., and weigh. A blank determination may be made with the reagents, but this has never been found to be positive. The method is regarded as giving results to an accuracy not greater than 1·5 per cent.

Where the α-glyceryl ether content is low, it is always advisable to use 5-ml. aliquots containing more than 50 mg. of unsaponifiable matter, and, owing to its greater solvent power, ethyl acetate is then the better solvent. The use of aliquots greater than 5 ml. introduces difficulties in adjusting the conditions. With many fats, as with batyl alcohol itself, agreement was found between the results of the two methods. Discrepancies with a number of fats indicate, however, the existence in their unsaponifiable fractions of substances other than α-glyceryl ethers that are attacked by periodic acid, and these instances are under fuller investigation.

A. O. J.

Assay for Choline Chloride in Pharmaceutical Products. W. C. Gakenheimer and R. M. Regnera (*J. Amer. Pharm. Assoc.*, 1946, **35**, 311–312)—The recent interest in the oral administration of choline for the treatment of certain diseases of the liver has led to the need for an accurate assay of choline chloride in pharmaceutical products. The use of phosphotungstic acid as a precipitant has been found to be rapid and reliable.

Method—(*a*) For liquid samples (solutions in water, elixirs, syrups, etc.)—Transfer such a volume of the sample as may be expected to contain about 1 g. of choline chloride to a 100-ml. volumetric flask and heat on a steam bath at a reduced pressure (100–125 mm. of Hg) to drive off moisture and any interfering decomposition products, such as ethylamine, that may be present. While the residue is still warm, shake it with 50 ml. of absolute alcohol, cool, and dilute to 100 ml. with the same solvent. Allow to settle, transfer 5 ml. of the clear supernatant liquid to a beaker containing 10 ml. of a filtered, 10 per cent. solution of phosphotungstic acid in absolute alcohol, digest the mixture on a steam bath for 5 min., and filter through a tared, sintered glass crucible of fine porosity. Wash the residue on the filter with 5 ml. of absolute alcohol, dry at 55° C. for 2 hr., and weigh. Multiply the weight of the precipitate by the factor 0·1375 to convert to choline chloride.

(*b*) For solid samples (tablets, capsules, etc.)—Extract an accurately weighed portion of the sample, expected to contain about 0·5 g. of choline chloride, in a micro-Soxhlet apparatus with 20 ml. of absolute alcohol for 3 hr. Transfer the extract quantitatively to a 50-ml. volumetric flask, distil off the alcohol, and heat the residue at 100° C. under reduced pressure for 15 min. as before. Dissolve in absolute alcohol, dilute to 50 ml. with the same solvent, and continue the assay as described above.

The factor given for converting the weight of precipitate to that of choline chloride is higher than the theoretical one of 0·1291 to compensate for the slight solubility of choline phosphotungstate in absolute alcohol under the assay conditions described. Different batches of phosphotungstic acid have been examined under the same conditions: the maximum variation in the weights of precipitate obtained was 0·7 per cent. and, although absolute alcohol was used in the experiments, it is stated that anhydrous denatured alcohol is satisfactory. No interfering substances have been encountered in several common vehicles tested. Results cited indicate that a satisfactory recovery of choline chloride is obtained when typical pharmaceutical products are submitted to the assay procedure.

J. A.

Biochemical

Micro-determination of Serine and Ethanolamine in Phospholipid Hydrolysates. C. F. Burmaster (*J. Biol. Chem.*, 1946, **165**, 1–6). The method depends on measuring the ammonia formed by the action of periodate on serine and ethanolamine by the micro-diffusion technique of Conway ("*Micro-diffusion Analysis and Volumetric Error.*" London, 1939).

Method—Put 1 to 5 ml. of 0·005 N-hydrochloric acid containing a few drops of mixed indicator (0·08 g. of methyl red and 0·02 g. of methylene blue dissolved in 100 ml. of 1 : 1 alcohol-water mixture and adjusted to a neutral grey colour with 0·005 N sodium hydroxide) into the central chamber of the diffusion cell and 1 to 5 ml. of the water-soluble extract from an acid hydrolysate of phospholipid into the outer chamber. The sample should contain from 0·05 to 0·3 mg. of total serine- and ethanolamine-nitrogen. Add 1 to 5 ml. of saturated potassium metaborate solution (prepared by mixing 61·9 g. of boric acid and 56·1 g. of potassium hydroxide in a mortar, and dissolving in 120 ml. of water), mix by rocking the cell, and place the cover on the cell, sliding it so as to cover all but a small portion of the outer chamber. Pipette 1 ml. of 0·05 M periodic acid through the small opening and quickly slide the cover shut. Mix the contents of the outer chamber by rotation and allow diffusion to proceed at room temperature overnight. Titrate the standard acid remaining in the central chamber to a neutral grey colour with 0·005 N-sodium hydroxide (carbonate-free). Carry out a similar procedure without the addition of periodate on a similar sample to serve as a "blank." The difference between the values for the sample and the "blank" is a measure of the serine- plus ethanolamine-nitrogen. The serine-nitrogen can be determined separately on another sample by the ninhydrin method. One ml. of 0·005 N-sodium hydroxide is equivalent to 0·007 mg. of "periodate nitrogen." Recoveries of serine and ethanolamine added to phospholipid hydrolysates were almost theoretical. F. A. R.

Chemical Estimation of Riboflavine. M. L. Scott, F. W. Hill, L. C. Norris, and G. F. Heuser (*J. Biol. Chem.*, 1946, **165**, 65–71)—The method of Hodson and Norris (*J. Biol. Chem.*, 1939, **131**, 621; ANALYST, 1940, **65**, 114) has been modified to eliminate interference by highly fluorescent non-riboflavine pigments. The major modification consists in the substitution of potassium permanganate oxidation of the interfering pigments for the reduction procedure previously used.

Method—To 1 to 5 g. of the finely ground sample in a 250-ml., red conical flask add 100 ml. of acid acetone solution (250 ml. of N-hydrochloric acid diluted to 1 litre with acetone). Heat the mixture gently under reflux for 1 hour, cool to room temperature, and adjust to *p*H 6·6 to 6·8 with trisodium phosphate solution. Dilute with water to 200 ml., leave for 5 min., and filter through a No. 12 Whatman fluted filter paper, discarding the first 10 to 20 ml. of filtrate. Pipette a 10- to 50-ml. portion of the filtrate into a 200-ml. volumetric flask, adjust the *p*H to 3·7 with glacial acetic acid, and add 2 ml. of 4 per cent. potassium permanganate solution for each 10 ml. of filtrate. Leave for 3 min., and then add 3 per cent. hydrogen peroxide until the colour changes from purple to light brown. Make up to volume, add 1 to 2 g. of Celite filter aid, and filter through a No. 44 Whatman filter paper, discarding the first 10 to 20 ml. of filtrate. Put 15 ml. of the remainder into the glass cell of a fluorimeter, and take the reading (A) after setting the galvanometer to zero with the aid of a distilled water "blank," and after recording the reading (S) given by a standard solution of riboflavine prepared as follows:—

First prepare a stock solution of riboflavine by dissolving 50 mg. in a litre of water containing a few drops of glacial acetic acid to stabilise the solution, and then mix 20 ml. of this aqueous solution with 100 ml. of acid acetone solution neutralised to *p*H 6·6 to 6·8 with trisodium phosphate solution, and dilute to 200 ml. with water. Adjust the *p*H of 40 ml. of this solution to 3·7 with glacial acetic acid, add 8 ml. of 4 per cent. potassium permanganate solution and, after 3 min., almost decolorise the solution by means of 3 per cent. hydrogen peroxide. Dilute to 200 ml., and filter through a No. 44 Whatman filter paper after adding Celite filter aid. Add 1 ml. of this standard riboflavine solution to 15 ml. of water in the fluorimeter cell and note the reading (S).

When the fluorescence (A) of the unknown solution has been measured, add to it 1 ml. of the standard riboflavine solution and again take a reading (B). Then reduce the riboflavine to the non-fluorescing leuco form by adding 1 ml. of sodium hydrosulphite solution prepared by dissolving 5 g. of $Na_2S_2O_4$ in 100 ml. of 0·2 M Na_2HPO_4, adding 1 to 2 g. of filter aid, and filtering through a No. 44 Whatman filter paper. Take a third reading (C). Finally, repeat the reading (S) with the standard solution alone to ensure that the light intensity has not changed during the above operations. The amount of riboflavine (μg. per g.) in the unknown solution is calculated from the expression:

$$\frac{A - (17\,C/15)}{(16\,B/15) - A} \times 0{\cdot}0625 \times \text{dilution factor}$$

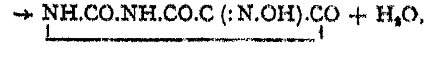
A comparison of the values obtained by this method for the riboflavine contents of a wide variety of foods and feeding stuffs with those obtained by the micro-biological method shows good agreement between the two methods. F. A. R.

Microbiological Estimation of Riboflavine in Blood. E. A. M. Bradford and H. Coke (*Biochem. J.*, 1945, **39**, 379–385)—Strong *et al.* (*J. Biol. Chem.*, 1941, **137**, 363) estimated riboflavine microbiologically in whole, haemolyzed blood. This on being autoclaved, however, produces a large amount of solid which makes the results somewhat variable. In the present method an acid extract of blood is prepared; this not only eliminates most of the solid but also gives a stable extract which can be stored overnight, if desired. It is pointed out that acid production after 72 hrs.' incubation at any level of riboflavine is a measure of the maximum population supportable in the medium at that level, whereas after shorter periods of incubation the population may not have reached the stable maximum, so that neither acid production nor opacity will be directly proportional to the riboflavine concentration. To obtain satisfactory results therefore: (*a*) time must be allowed for the population to reach a stable maximum; (*b*) the size of the original inoculum must be standardised; and (*c*) the culture should be in a certain relatively deficient state.

Mix 1 ml. of venous blood, immediately after withdrawal, with 4 ml. of 0·1 N hydrochloric acid in a conical centrifuge-tube. Plug with cotton-wool, autoclave at 15 lb. pressure for 15 mins., cool and store in the dark until required. Add 0·4 ml. of N sodium hydroxide, neutralise to *p*H 4·5 with 0·02 N acetic acid and centrifuge. Transfer the supernatant liquid to a 10-ml. volumetric flask, add 2 ml. of water to the residue, shake, centrifuge and add the washing to the flask. Wash with another 2 ml. of water, again add the washing to the flask, adjust to *p*H 6·7 with 0·1 N sodium hydroxide and dilute to 10 ml. Centrifuge if necessary. Assay 2-ml. portions of the extract in duplicate by the method of Strong *et al.*, inoculating each tube with one drop of a 24 hr.-old culture introduced by means of a 1-ml. syringe fitted with a No. 10 needle from which the oblique tip has been cut. To prepare the culture, inoculate the liquid medium directly from a stab-culture, incubate for 24 hr., centrifuge and re-suspend the cells in 10 ml of sterile saline. Check the opacity by means of a Spekker photo-electric absorptiometer. With these precautions, the standard curve should be linear. The average error with 25-μg. quantities of riboflavine per 100 ml. was estimated to be ±8%. By destroying the riboflavine in blood by irradiation, it was shown that the riboflavine-free blood did not stimulate the growth of the organism at any level but that, when added to known amounts of riboflavine, it exerted a marked inhibitory effect if more than 0·2 ml. was used. A method of removing this inhibitory substance has not been yet devised.

F. A. R.

Method for the Determination of Alloxan. G. Brückmann (*J. Biol. Chem.*, 1946, **165**, 103–113)—Alloxan in amounts ranging from 0·04 to 0·4 mg. can be determined by a method based on the following principle: (*a*) conversion to violuric acid by evaporation *in vacuo* with hydroxylamine



$$\mathrm{NH.CO.NH.CO.CO.CO} + \mathrm{H_2N.OH} \rightarrow \mathrm{NH.CO.NH.CO.C\,(:N.OH).CO} + \mathrm{H_2O},$$

followed by (*b*) photometric estimation of the violuric acid in the form of its ferrous complex

$$3\ C_4H_3N_3O_4 + FeSO_4 \rightarrow Fe(C_4H_2N_3O_4)_2.C_4H_3N_3O_4 + H_2SO_4.$$

Method—Put the sample into a 50-ml. distillation flask, fitted with a fine capillary reaching exactly to the bottom, and immerse the flask in a glycerol bath. Add 1 ml. of hydroxylamine solution, prepared by dissolving 15 mg. of the hydrochloride per ml. in a mixture of equal volumes of glycerol and absolute alcohol, and add sufficient alcohol to bring the volume to about 20 ml. Heat the bath to 40 to 50° C. and begin distillation with the aid of a water pump, raising the temperature gradually over a period of about 12 mins. to 90 to 100° C., and then within the next 5 mins., to 110 to 120° C. Allow the flask to cool in air under the reduced pressure, and then add exactly 4·5 ml. of absolute alcohol followed by 0·2 to 0·3 g. of solid quinine hydrochloride. Shake thoroughly for 1 min. to ensure complete solution, and finally add 0·1 ml. of a solution prepared by adding 3 drops of water and 5 ml. of 1 : 1 glycerol - alcohol mixture to 25 mg. of ferrous ammonium sulphate. Filter the blue solution, if necessary, and evaluate the colour in a photometer at 610 mμ. Calculate the results from a standard curve prepared from pure alloxan monohydrate. Alternatively, violuric acid can be used as a convenient and stable standard, but then the efficiency of the condensation procedure should be checked by comparing the calibration curve from an alloxan standard with that obtained directly from violuric acid. Each milligram of alloxan monohydrate should give the same colour as 0·97 mg. of violuric acid.

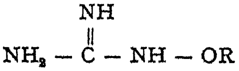
All N-substituted alloxans yield blue iron violurates by this method, whilst dialuric acid and alloxantin also behave in the same way as alloxan. No colour is produced by alloxanic acid, parabanic acid, murexide, uric acid, barbituric acid, or phenobarbitone. Ninhydrin gives a brown colour.

Contrary to expectations, Folin-Wu or trichloroacetic acid extracts of biological materials were unsatisfactory, as these filtrates contain interfering substances. Better results were obtained by treatment with ammonium sulphate, alcohol, and fuller's earth.

Procedure—Grind 1 g. of tissue with 0·5 g. of ammonium sulphate and extract the mixture with successive portions of ice-cold, absolute alcohol, using a total of 20 ml. Centrifuge and shake the supernatant liquid with 1 g. of fuller's earth after adding 2 drops of *N* hydrochloric acid. Again centrifuge, and use the clear liquor for the colour development. In a similar way, shake 1 ml. of blood containing anticoagulant with 0·5 g. of ammonium sulphate, and then with 20 ml. of cold, absolute alcohol. Centrifuge and shake the supernatant liquid with fuller's earth, and continue the procedure recommended for the estimation of alloxan in tissue. The recovery of alloxan from alcoholic tissue extracts was 70 to 90 per cent. of the theoretical, but recoveries from the tissues themselves were very much lower owing to the rapidity with which alloxan is destroyed by the tissues. F. A. R.

Colorimetric Estimation of Canavanine. R. M. Archibald (*J. Biol. Chem.*, 1946, 165, 169–178)

—Canavanine reacts with a solution of sodium nitroprusside that has been exposed to sunlight to form a ruby-coloured solution. This reaction appears to be specific for substances of the type



$$NH_2 - \overset{\overset{\displaystyle NH}{\|}}{C} - NH - OR$$

and has been made the basis of a simple quantitative method of estimating canavanine.

Procedure—To 1 ml. of the neutralised unknown solution containing up to 0·25 mg. of canavanine, add 0·5 ml. of *M* phosphate buffer solution of *p*H 7·2 (34·5 g. of $NaH_2PO_4.H_2O$ and 130·6 g. of K_2HPO_4 in 1 litre), and 0·5 ml. of a nitroprusside reagent prepared by mixing 0·5 ml. of 20 per cent. potassium carbonate solution and 0·4 ml. of 30 per cent. hydrogen peroxide with 10 ml. of 2 per cent. sodium nitroprusside solution; after standing for 30 mins. at room temperature, this reagent, which should be prepared daily, is ready for use. Put into test tubes 0·2-, 0·5-, 0·8-, and 1·0-ml portions of a solution containing 0·25 mg. of canavanine per ml., and dilute each solution to 1·0 ml. with water. Put 1 ml. of water into another tube to serve as a reagent "blank," and add phosphate buffer and nitroprusside reagent to each tube. Leave the solutions for 2 hours in the dark, and then evaluate the colours at 520 mμ, after adjusting the instrument to zero by means of the blank. Calculate the canavanine content of the unknown from the curve obtained by plotting the optical density of the standard solutions against their concentrations.

The method gives satisfactory results with pure solutions; blood filtrates and urine contain unidentified substances that inhibit the development of the colour, so that in its present form the method is not applicable to these materials. It appears to be applicable to some plant extracts, however. The blue colours given by thiourea and other organic sulphur compounds are unstable, and are not likely to interfere with the application of the method. F. A. R.

Hydrolysis Procedures for the Estimation of Tryptophan in Proteins and Foodstuffs by the Micro-biological Procedure. I. T. Greenhut, B. S. Schweigert, and C. A. Elvehjem (*J. Biol. Chem.*, 1946, 165, 325–332)

—The liberation of tryptophan from proteins and foodstuffs by acids, alkalis, and enzymes has been investigated. The most satisfactory recoveries were obtained by incubation with a mixture of pancreatin and hog mucosa. Pancreatin alone gave wide variations in the amount of tryptophan liberated, and the addition of hog mucosa did not eliminate this variability if the solutions were not shaken. If, however, the samples were shaken continuously, the liberation achieved in one day was as great as that achieved in 5 days without shaking, and the variability between samples was almost eliminated.

Procedure—Put a 1-g. sample of animal tissue or 100- to 200-mg. sample of protein into a 100-ml. volumetric flask, add 50 mg. of pancreatin, and 25 mg. of hog mucosa. Dilute with water to approximately 50 ml., and adjust the *p*H to 8·2 with 0·1 *N* sodium hydroxide. Cover the solution with toluene, and incubate at 37° C. with continuous shaking for one day. The digest can be used for micro-biological assays by the recognised procedure. F. A. R.

Determination of Sulphonamides in Tungstic Acid (Folin - Wu) Blood Filtrates. E. G. Schmidt (*J. Lab. Clin. Med.*, 1946, 31, 694–699)

—The determination of blood urea, non-protein nitrogen, sugar, etc., and of the unconjugated sulphonamides is often required for the same sample of blood. Routine blood filtrates generally contain tungstate ions, derived from the deproteinisation procedure, which interfere with the colorimetric determination of sulphonamides by the method of Bratton and Marshall (*J. Biol. Chem.*, 1939, **128**, 537). A modification of this test is described wherein the effect of the tungstate ions is obviated.

Method—Haemolyse the sample of oxalated blood by mixing 1 volume with 8 volumes of 0·0877 *N* sulphuric acid, adding 1 drop of *N* sulphuric acid as a precaution against undue alkalinity of the potassium oxalate, and precipitate the proteins with 10 per cent. sodium tungstate solution in the usual way. To 5 ml. of the deproteinised blood filtrate add 5 ml. of a 15 per cent. aqueous solution of *p*-toluene sulphonic acid monohydrate containing 2 per cent. of citric acid, and 1 ml. of a 0·1 per cent. solution of sodium nitrite. Leave for 3 min. and add 1 ml. of a 0·1 per cent. aqueous solution of *N*-(1-naphthyl)-ethylenediamine dihydrochloride. Compare the red colour produced with that of the appropriate standard treated in the same manner in a Duboscq-type colorimeter. If a photo-electric instrument is used, the method is modified as follows. Add 5 ml. of the *p*-toluene sulphonic acid mixture, previously diluted with an equal volume of water, or of the citric acid - sulphuric acid solution, similarly diluted, to 2 ml. of the blood filtrate, 2 ml. of the appropriate standard solution, and 2 ml. of distilled water, respectively. Then add 0·5 ml. of 0·1 per cent. sodium nitrite solution to each, followed, after 3 min., by 0·5 ml. of an aqueous solution containing 0·5 per cent. of ammonium sulphamate and 13·8 per cent. of sodium dihydrogen phosphate ($NaH_2PO_4.H_2O$). Leave for a further period of 3 min. and add to each 0·5 ml. of *N*-(1-naphthyl)-ethylenediamine dihydrochloride solution. Set the photo-electric instrument at zero with the water blank and then compare the colour produced by the test with that of the standard; a green filter (No. 54) should be used. The standard solutions are prepared by dissolving 250 mg. of the sulphonamide (or 1 g. if sulphanilamide) in 1 litre of 0·0877 *N* sulphuric acid and diluting these stock solutions to form working standards of the required concentration before use. The *p*-toluene sulphonic acid - citric acid solution prevents the formation of a violet tint due to the presence of tungstate ions; except with sulphathiazole, it is possible to use a 2 per cent. solution of citric acid in 0·2 *N* sulphuric acid in place of the more expensive *p*-toluene sulphonic acid mixture. It is stated that except with sulphanilamide, which gives an orange-red colour in the test, the tints produced by the various sulphonamides match each other reasonably well, and that only one standard, therefore, need be prepared, conversion factors being determined experimentally by comparative analyses. Since it is known that sulphonamides are, in some degree, adsorbed by the protein precipitate during the preparation of blood filtrates (Schmidt, *J. Biol. Chem.*, 1938, **122**, 757; *J. Lab. Clin. Med.*, 1939, **24**, 982; Scudi, *J. Biol. Chem.*, 1938, **122**, 539), percentage recoveries have been determined and found to be consistent and independent of the initial concentration of the drug in the blood. At 26° C., the figures obtained are: sulphanilamide, 88·3%; sulphadiazine, 75·7%; sodium sulphadiazine, 75·8%; sulphamerazine, 75·8%; sulphamethazine, 76·1%; sulphapyridine, 74·4%; sulphathiazole, 56·7%; and sulphacetamide, 83·8%. These values are slightly higher (from 2 to 6%) at 33° C., and slightly lower (from 0·5 to 2%) at 18° C. Comparison of the results obtained on whole blood by the method of Bratton and Marshall (*loc. cit.*) and the proposed modification indicates that agreement is satisfactory. J. A.

Polarographic Estimation of Steroid Hormones. Polarography of Neutral 17-Ketosteroids in Urinary Extracts. J. Barnett, A. A. Henly, and C. J. O. R. Morris (*Biochem. J.*, 1946, **40**, 445–449).—Wolfe *et al.* (*J. Biol. Chem.*, 1940, **136**, 653) showed that certain ketosteroids condensed with the Girard T reagent to give water-soluble hydrazones, which under suitable conditions yielded well-defined polarographic waves. The wave span was proportional to the steroid concentration over a considerable range and amounts of the order of 10 µg. could be estimated with accuracy. This wave appears to be specific for 17-ketosteroids. The method was later extended (*J. Biol. Chem.*, 1941, **140**, 215) to hydroxysteroids, which were oxidised to the corresponding ketones.

In applying this method to the estimation of 17-ketosteroids in urinary extracts, it was found that the form of the wave was distorted because, (*a*) the final discharge occurred at a lower voltage, so that the point of inflexion of the wave was difficult to distinguish, and (*b*) a second small wave extending from about −1·0 to −1·3 v. made it difficult to establish a true base line. The interfering substance was ketonic in nature, since extracts not treated with Girard T reagent gave no polarographic wave. The difficulties arising from the presence of this impurity were overcome (*a*) by decreasing the rate of rise of applied voltage as suggested by Werthessen and Baker (*Endocrinology*, 1945, **36**, 351), and (*b*) by destroying a considerable proportion of the impurity by oxidation with potassium permanganate in aqueous dioxan.

Heat a portion of the urine, estimated to contain 0·5 to 1 mg. of ketosteroids, rapidly to boiling and add 15 ml. of conc. hydrochloric acid per 100 ml. of urine. Continue boiling under reflux for exactly 10 mins., cool immediately and add 26 g. of sodium chloride. Extract with four 50-ml. portions of benzene, wash the combined extracts twice with a 9% solution of sodium carbonate and evaporate under reduced pressure to slightly less than 100 ml. Transfer to a separating funnel and rinse out the flask with sufficient benzene to bring the total volume to 100 ml. Remove phenols by shaking with four 25-ml. portions of 2 *N* sodium hydroxide and then shake with 25 ml. of a freshly prepared 10% solution of sodium hyposulphite in 2 *N* sodium hydroxide for 3 mins., keeping the separator stoppered the whole time. Finally wash with four 25-ml. portions of water and distil the colourless benzene extract to dryness under reduced pressure, first in a bath at about 60° C. and finally at room temperature. Dissolve the residue in 1·0 ml. of pure benzene. This is the *crude extract*.

To purify this crude extract, evaporate 0·1 ml. to dryness under reduced pressure and dissolve the residue in 0·5 ml. of freshly redistilled dioxan, add 0·1 ml. of 0·1 *N* potassium permanganate solution and 0·25 ml. of 0·01 *N* periodic acid, stopper the tube and leave it at room temperature for 15 mins. Transfer the solution to a separating funnel, rinsing out the tube with 5 ml. of benzene and 5 ml. of water. Shake and discard the lower layer. Shake the benzene solution with 3 ml. of a freshly prepared 10% solution of sodium hyposulphite in 2 *N* sodium hydroxide and then four times with water. Evaporate the benzene extract to dryness. This is the *oxidised extract*.

To prepare the Girard T hydrazones, evaporate 0·1 ml. of the crude extract to dryness under reduced pressure at 30° C. and to the residue, or to the total residue from the oxidised extract, add 0·02 ml. of a freshly prepared solution containing 20 mg. of Girard T reagent in 0·2 ml. of pure acetic acid. Stopper the tube and heat in a boiling water bath for 2 mins. Cool, add 2 ml. of the base solution (a 1:2:1 mixture of 0·5 *N* sodium chloride, 0·2 *N* sodium hydroxide and water) and transfer the clear solution to the polarographic cell. Add about 1 ml. of mercury and pass a rapid stream of nitrogen through the solution for 3 mins. Insert the dropping electrode and measure the current in the usual way over a potential range of −0·9 to −1·8 volts.

F. A. R.

Photometric Determination of Total Cholesterol in Plasma or Serum by a Modified Liebermann-Burchard Reaction. A. Saifer and O. F. Kammerer (*J. Biol. Chem.*, 1946, **164**, 657–677)—The new method differs from those previously described in that no attempt is made to remove the water from the biological fluid; instead an amount of water, equivalent to that present in the biological fluid, is added to the cholesterol standards. In the course of the reaction the water present in the standard or unknown is converted into acetic acid. It is well-known that the Liebermann-Burchard reaction takes place in acetic acid solution as well as in chloroform, although the rate of reaction varies with the nature of the solvent.

Pipette duplicate 0·20-ml. samples of clear unhaemolysed serum or plasma into test tubes containing 5 ml. of acetic anhydride - dioxan mixture (3:2). Mix well and heat in a boiling water bath for 30 mins. with occasional shaking. Filter the solution through a Whatman No. 42 filter paper into graduated colorimeter tubes. Rinse the original tubes with 0·25-ml. portions of the acetic anhydride - dioxan mixture, filter, combine the filtrate and washings, and adjust the final volume to 5·00 ml. To each tube add 0·25 ml. of conc. sulphuric acid from a micro-burette, mix well and, exactly 5 mins. later, immerse the tube in a water bath maintained at 37·5° C. Five mins. later, remove the tube and evaluate the colour in a photoelectric colorimeter with a 650 mμ filter against a reagent blank set at 100% transmission. Take readings at 5-minute intervals until the maximum density value is reached. Calculate the cholesterol content corresponding to this maximum density from a standard curve prepared from solutions containing 0·2 to 1·2 mg. of cholesterol and 0·18 ml. of water. A more satisfactory standard for use in the assay of plasma or serum is made by dissolving 25 mg. of cholesterol and 27·7 mg. of cholesteryl acetate in 240 ml. of the acetic anhydride - dioxan mixture by heating, cooling to room temperature and diluting to 250 ml. with the solvent mixture. The recoveries of cholesterol, cholesterol ester or mixtures of both added to normal human serum were almost theoretical.

F. A. R.

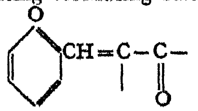
Activated Glycerol Dichlorohydrin. A New Colorimetric Reagent for Vitamin A. A. E. Sobel and H. Werbin (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 570–573)—Recently (*J. Biol. Chem.*, 1945, **159**, 681; ANALYST, 1946, **17**, 43) the authors proposed the use of glycerol 1:3-dichlorohydrin as a new reagent for the quantitative estimation of vitamin A. It has since been found that some commercial samples of the dichlorohydrin do not give colour when added to vitamin A. By distilling these with 1 to 5% of antimony trichloride at 4- to 40-mm. pressure, a reagent was obtained which reacted satisfactorily with vitamin A. Activation could also be obtained by addition of conc. hydrochloric acid, acetyl chloride, phosphorus pentachloride, anhydrous aluminium chloride and benzoyl chloride, but conc. sulphuric acid, zinc chloride and stannic chloride caused the reagent to give blue colours that did not change to violet. Glycerol 2:3-dichlorohydrin and glycerol 1:3-dibromohydrin when activated gave blue colours changing to violet. The concn. of antimony (as antimony trichloride) in the activated reagent varied from a trace to 0·67%, but the activation is not due to antimony trichloride *per se*, since glycerol dichlorohydrin to which 0·1 to 1·0% of antimony trichloride had been added was inactive towards vitamin A. Although the activated reagent and the original reagent react in a similar manner towards vitamin A, the activated reagent behaves differently towards carotene and various sterols. A spectrophotometric study was made of the violet colour produced by the activated reagent with vitamin A in chloroform soln. The colour was found to be stable for 2 to 10 min. after addition of the reagent at 555 mμ, the wavelength of maximum absorption, and the colour obeys Beer's law over a wide range. With the Beckman Quartz Spectrophotometer with a band width of 5 mμ or less, the extinction coefficient of the coloured liquid produced by the activated reagent with crystalline vitamin A alcohol was 1270 at its wavelength of maximum absorption, *viz.*, 553–556 mμ. The corresponding coefficient for the crystalline acetate was 1350 calculated for its vitamin A content. The lower extinction of the alcohol was subsequently shown to be due to some impurity in the vitamin alcohol. With the Beckman spectrophotometer a nearly linear relation was found between the optical density and vitamin A concns. ranging from 1·36 to 13·63 μg. per 5 ml., the average extinction being 1300 ± 24. With the Coleman Universal Spectrophotometer with its 35-mμ band width a linear relation existed within a smaller concn. range, *viz.*, 1·36 to 5·5 μg. per 5 ml., the average extinction being 1120 ± 15.

The interference of carotene, vitamin D$_2$ and other related sterols in the estimation of vitamin A with the activated reagent was investigated by measuring the absorption of the coloured solns. produced under the conditions of the vitamin A estimation. Except with carotene the interference was negligible. The error due to an equal wt. of carotene was 11% with the Coleman spectrophotometer and 7·35% with the Beckman instrument. This may be compared with a 5% interference in the antimony trichloride method. The carotene interference may be evaluated by the method of Dann and Evelyn (*Biochem. J.*, 1938, **32**, 1008) with the activated reagent just as with antimony trichloride. The absorption curves of crystalline vitamin A acetate and carotene treated with the reagent show that above 630 mμ the vitamin A product no longer absorbs light, whereas the carotene product continues to do so beyond this point. Thus by taking readings at two wavelengths, *viz.*, 555 and 800 mμ, both carotene and vitamin A can be evaluated, the optical densities being additive.

To prepare the reagent add 100 ml. of chloroform containing 10 to 50 g. of dissolved antimony trichloride (prepared without "anhydrous" precautions) to 1000 ml. of glycerol dichlorohydrin. Distil the mixture under reduced pressure (4 to 40 mm.) in an all-glass apparatus. Discard the chloroform fraction and collect and store the product in a glass-stoppered bottle at room temp. Some samples of dichlorohydrin required two distillations to produce a colourless reagent. To develop the colour add 1 ml. of chloroform containing the desired amount of vitamin A or carotene, or both, to 4 ml. of activated reagent, shake the mixture thoroughly and maintain it at 25° C. in a water bath until ready for the spectrophotometer, the colour being stable for 8 min. The colour is not affected by water present in a humid atmosphere, and the reagent does not leave white deposits of antimony oxychloride in the photometer cells. A. O. J.

Organic

The Specificity of the Salicylaldehyde Reaction for the Detection of Acetone. T. Thomson (*J. Soc. Chem. Ind.*, 1946, **65**, 121–124)—Braunstein's theory (*Nature*, 1937, **140**, 427) of the mechanism of the salicylaldehyde reaction for detecting acetone is rejected, and it is shown that the reaction is less selective than was stated by Braunstein. The reaction evidently involves condensation of the salicylaldehyde with a methylene group in the α-position to an unsaturated group such as carbonyl, and the formation of an alkali salt of the resulting resonating structure:



Method—For the test, use aqueous solutions; dissolve in alcohol substances insoluble in water, and dilute with water. Place in a test tube 5 ml. of the solution under examination, and add 4 ml. of 40 per cent. sodium hydroxide solution, followed by 1 ml. of reagent prepared by diluting 20 ml. of salicylaldehyde to 100 ml. with 95 per cent. alcohol. Heat the reactants at 40 to 50° C. for 20 mins., and compare the colour with that of a "blank" carried out simultaneously on the reagents. The following results were obtained.

Substance	Concentration of soln. used for test (parts by volume)	Colour obtained in test
Acetone	1 in 50,000	Red
,,	1 in 250,000	Red with yellowish tinge
,,	1 in 6,250,000	Pale orange; definitely deeper colour than reagents alone
,,	1 in 12,500,000	Lemon-yellow, indistinguishable from reagents alone.
Pyruvic acid	1 in 50,000	Red with yellowish tinge
,, ,,	1 in 250,000	Pale orange, definitely deeper than reagents alone
Ethyl acetoacetate	1 in 200	Orange-red
,, ,,	1 in 500	Brownish-yellow
,, ,,	1 in 250,000	Golden-yellow, just distinguishable when compared directly with lemon-yellow colour of reagents
Acetophenone	1 in 200	Bright red
,,	1 in 100,000	Lemon-yellow, indistinguishable from reagents
Benzalacetone	1 in 250,000	Golden-yellow, just distinguishable when compared directly with lemon-yellow colour of reagents
Acetaldehyde	1 in 500	Golden-yellow, just distinguishable when compared directly with lemon-yellow colour of reagents
Propionaldehyde	1 in 500	Deep orange
,,	1 in 125,000	Lemon-yellow, indistinguishable from reagents
Methyl ethyl ketone	1 in 500	Orange
,, ,, ,,	1 in 50,000	Golden-yellow, deeper than reagents
Diethyl ketone	1 in 500	Golden-yellow, decidedly deeper than reagents
*Cyclo*hexanone	1 in 500	Deep orange
,,	1 in 250,000	Yellow, barely distinguishable from reagents
Methyl *cyclo*hexanone	1 in 500	Orange
,, ,,	1 in 50,000	Lemon-yellow, indistinguishable from reagents
Cyanacetic acid	Approx. 3 in 100	Deep orange
Fluorene	—	No reaction

The constitution of the coloured products has been examined. That formed with acetone is the monosodium salt of *o*-hydroxybenzalacetone (salicylacetone); the neutral material precipitated from the aqueous solution on titration with acid had, after recrystallisation from aqueous alcohol, m.p. 137° to 139° C. The product formed with benzalacetone is the monosodium salt of salicylbenzalacetone. Ethyl acetoacetate gives the sodium salt of salicylacetone, evidently formed by normal ketonic hydrolysis from the condensation product of salicylaldehyde and ethyl acetoacetate. Acetophenone gives the sodium salt of salicylacetophenone; the neutral material obtained after acidification of the salt had m.p. 154° to 156° C. E. M. P.

Analytical Reactions with Organic Reagents. A. Steigmann (*J. Soc. Chem. Ind.*, 1946, 65, 233–234)—The following specific or very selective reagents are described. (1) *A sensitive reagent for thiosulphate*—By heating, dissolve 0·1 g. of thionine (B.D.H. redox indicator) in 50 ml. of ethylene glycol or other inert alcohol. Add 1 ml. of this solution to 50 ml. of water and dilute to 100 ml. To 3 ml. of the carbon-filtered and then evaporated wash water of the gelatin to be tested, or other test solution, add 1 ml. of the diluted thionine solution. Perform a "blank" test. To the test and the "blank," add 0·4 ml. of 5 N sulphuric acid. Stand both in boiling water for 7 to 8 min., and cool in running cold water for 20 min. Less than 1 : 40,000 sodium thiosulphate causes a colour change from violet to blue, whilst higher concentrations cause partial or total bleaching. The new test is less sensitive, but much more selective than the iodine-azide reaction. Only thiocarbamides interfere at 50 to 100 times higher concentrations by giving direct bleaching without prior colour change.

(2) *Specific reagents for nitrate, guanidine, urea, and nitroso-compounds*—(*a*) Add 1 g. of carbazole to 100 ml. of *iso*propyl alcohol, followed by 1·4 g. of dimethylglyoxime. Dissolve by heating.

Crystals precipitate on cooling, and are dissolved again before use. To 3 ml. of concentrated hydrochloric acid add 0·5 ml. of 1 per cent. arginine hydrochloride, or guanidine carbonate solution, or 1 ml. of 10 per cent. gelatin solution (as a source of arginine), and then 0·2 ml. of the reagent, followed by 1 to several drops of the test solution, or of water for the "blank." Heat for 20 min. in boiling water. Traces of nitrate produce a brilliant blue coloration. By using a small amount of nitrate in the reagent instead of guanidine, the reagent can be used to test for guanidine (arginine). To 3·5 ml. of concentrated hydrochloric acid add 1 ml. of 2 per cent. gelatin solution, 2 drops of 1 per cent. potassium nitrate solution, and 0·5 ml. of the reagent. Boil for 12 min. By dissolving in 10 ml. of *iso*propyl alcohol the precipitate from an excess of carbazole, the blue colour goes into the aqueous solution, which can then be used for colorimetric determination of nitrate.

(*b*) To 3 ml. of concentrated hydrochloric acid add 0·5 ml. of 0·5 per cent. alcoholic acetyl benzoyl solution, 0·2 ml. of 10 per cent. urea solution, and 1 ml. of nitrate test solution. Boil for 10 min. Nitrates cause the development of a stable, carmine colour, suitable for colorimetric determinations. Guanidines (arginine) do not interfere. The reaction is specific for ureas (citrulline) when, instead of urea, nitrate is included in the reagent. To detect citrulline in gelatin, add 2 drops of 1 per cent. potassium nitrate solution, and 0·5 ml. of 0·5 per cent. alcoholic acetyl benzoyl solution to 3 ml. of concentrated hydrochloric acid, followed by 0·5 ml. of water and 1 g. of gelatin powder. Boil for 6 min. The reagent cannot be used for proteins containing tryptophan and sugars, or giving a strong humin reaction on acid hydrolysis. A slight humin reaction is given by gelatins, but this does not interfere seriously.

(*c*) Cheaper reagents for nitrate or urea are obtained by substituting dimethylglyoxime or diacetylmonoxime for acetyl benzoyl in (*b*). The reactions for nitrate described above are not interfered with by traces of nitrite, and are as sensitive as the nitrate reactions with polyhydric phenols.

Resorcinol, a 1 per cent. solution in concentrated hydrochloric acid, is also a reagent for nitroso-compounds, such as α-nitroso-β-naphthol and nitroso-R-salt. Add 3 ml. of the resorcinol solution to the evaporated test solution; transfer to a test tube, and put into boiling water, whereupon there develops a blue-violet colour which, after dilution with water, can be extracted with ether. The colour in ether is red. Resorcinol in concentrated sulphuric acid reacts also with some nitro-compounds, *e.g.*, with *p*-nitrophenylhydrazine, but not with 2 : 4-dinitrophenylhydrazine, giving sulphonated dyes which are not extracted by ether. The dyes from the resorcinol nitrate and nitrite reactions are also soluble in ether. Resorcinol cannot be used for gelatin in Selivanov's sugar reaction, as technical gelatins invariably contain traces of nitrates, or nitrites, or both.

(3) *Reagents for carbohydrate-sugar*—(*a*) Dissolve 80 g. of urea in 200 ml. of concentrated hydrochloric acid. (*b*) Dissolve 0·2 g. of tryptophan in 100 ml. of concentrated, metal-free hydrochloric acid; use the reagent fresh and, if suitable, sensitised by 50 mg. of cysteine hydrochloride. To 0·5 g. of gelatin powder, or the dry residue of an evaporated carbohydrate or sugar solution, add 3 ml. of solution (*a*) or (*b*). Put the test tubes into boiling water for 20 min. or 3 min., respectively, soaking the gelatin for 10 min. in the reagents before heating A brown colour (melanin) develops with (*a*), and a violet colour with (*b*), if carbohydrates or sugars are present. Results that have been obtained with gelatins of different kinds are noted. Carbohydrate solutions will indicate tryptophan, *e.g.*, a solution of 1 g. of potato starch in 100 ml. of concentrated hydrochloric acid. Raise the temperature of the solution to 70° C., cool to room temperature, and use as a tryptophan (Voisenet) reagent. (*c*) Boiling monochloroacetic acid is a reagent for protein sugars. To 2 g. of monochloroacetic acid add 25 to 100 mg. of dry protein, and raise the temperature to 185° C. (b.p. of the acid). Reflux for 5 min., add 2 ml. of water, and carry out a colorimetric estimation. Hydroxyproline or tryptophan condenses in boiling monochloroacetic acid with aldehydes to give melanin brown or violet colorations, respectively. If tryptophan and hydroxyproline are absent from the protein under test, add 5 to 10 mg. of one of these before the sugar test (this addition is unnecessary with gelatins). This test can also be used to detect tryptophan or hydroxyproline if only one of these is present. For example, to test gelatin for hydroxyproline, add 7 mg. of glucose and 10 to 20 mg. of the gelatin powder to 2 g. of chloroacetic acid. Heat under reflux at 185° for 5 min., dilute with 2 ml. of water, and estimate colorimetrically.

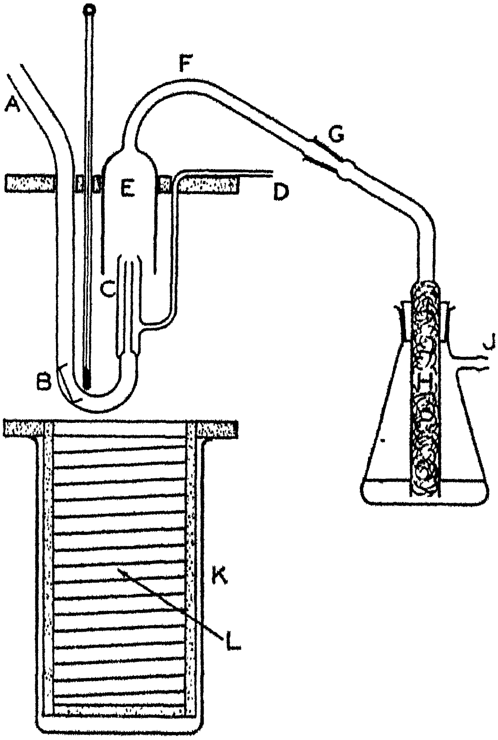
(4) *Anionic and cationic wetting agents and anionic and cationic "gelatins" as analytical reagents*—(*a*) *Reagent for alkaline earths*—To 100 ml. of 1 : 10,000 thionine solution add 2 ml. of B.D.H. Wanklyn's volumetric soap solution and 75 ml. of 0·880 ammonia. To 2 ml. of this reagent add 1 drop of the test solution, *e.g.*, tap water. If the solution contains an alkaline earth, the blue violet colour changes to red-violet. As the ammonia evaporates on keeping the solution, the reagent must not be too old. (*b*) *Reagent for soap*—To 100 ml. of 1 : 10,000 thionine solution add 75 ml. of 0·880 ammonia. Traces of soap, fat or soap-forming fatty acid change the red-violet colour of the reagent to blue-violet. (*c*) *"Metal acid" reagent*—To 100 ml. of 1 : 5,000 tropæolin 00 solution (or thymol blue solution) add 2 ml. of 2 per cent. Sapamin KW 200 per cent. solution, and then 200 ml. of 0·5 *N* hydrochloric acid diluted with 100 ml. of distilled water. Traces of sodium tungstate or salts of other metal acids turn the yellow reagent red. Cetyltrimethylammonium bromide (Cetavlon) can be used instead of Sapamin. (*d*) *Preparation of cationic "gelatin" and a cobalt-adsorbing reagent therefrom*—Stir 30 g. of air-dried gelatin powder, practically metal-free, into 85 ml. of acetic anhydride; raise the temperature within 20 min. to 125° while stirring. Add 150 ml. of water. Collect the acetylated gelatin on a Buchner funnel and wash several times with water. Dry the gelatin powder. To 100 ml. of 0·1 per cent. nitroso-R-salt solution add 7 g. of the cationic acetylated gelatin and 0·3 ml. of 5 *N* acetic acid. Heat to 50°, and stir occasionally. After 2 hrs., collect the stained gelatin powder on a Buchner funnel. Wash with water and dry. Use the powder as a sensitive cobalt-adsorbing reagent in the hot or boiling test solution (Schmidt, "*The Chemistry of the Amino Acids and Proteins*," Springfield and Baltimore, 1944, 720–778). (*e*) *Preparation of an anionic gelatin, for the adsorption of basic dyes and ammines (cationic complexes) of silver, copper, nickel, cobalt, cadmium, and zinc from ammoniacal solutions*—Stir 30 g. of gelatin powder into a mixture

of 10 ml. of 40 per cent. formaldehyde and 50 ml. of *iso*propyl alcohol. Heat to 80° C., and maintain this temperature for 15 min. Decant the excess of solution. Add 100 ml. of water to the tanned gelatin powder and collect it on a Buchner funnel. Wash several times with water and then with diluted ammonia (1 + 9). Wash again, filter, and dry. *Example of a metal adsorption*—Add to 3 ml. of 1 : 200,000 cobaltous nitrate solution 20 mg. of anionic gelatin powder and 2 drops of 5 *N* ammonia solution. Heat and shake, centrifuge, wash, and centrifuge again. Then add 2 drops of 0·5 per cent. *s*-benzylthiuronium chloride solution and 2 ml. of water to the centrifuged gelatin powder containing the cobalt. Heat, add 2 drops of 5 *N* ammonia solution, and heat again to produce the characteristic mauve colour of cobalt on the gelatin grains. Adsorbed nickel is detected in the same way. The procedure is the same for adsorption of the other metals mentioned above. E. M. P.

Analysis of Organoselenium Compounds. J. D. McCullough, T. W. Campbell, and N. J. Krilanovich (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 638–639)—Usual methods are either long or inaccurate. In testing available methods, the best results were obtained by a modification of the oxygen-combustion method at a glowing platinum surface (Niederl and Niederl, "*Micromethods of Quantitative Organic Analysis*," 2nd Ed., p. 208, New York, John Wiley and Sons, 1942). Van ter Meulen's method of determining selenium dioxide iodimetrically has been modified to eliminate errors due to the presence in a sample of hydrochloric acid or of chlorine; the length of the method, and the need for constant attention rendered new procedures desirable. These now are (*a*) Flame combustion giving the selenium content directly. (*b*) Volumetric methods yielding the equivalent weight of the compound.

Flame combustion method—This is based on a known method of halogen determination, but an oxygen flame is necessary for complete oxidation of the selenium to its dioxide.

Apparatus and Procedure—The apparatus used is shown approximately a quarter the actual size, ABCD being made of transparent quartz tubing, the dimensions of which are apparently not critical, and the chimney and absorption tube EFGH is of Pyrex glass with a T joint at G. H is packed with grease-free, clean Pyrex glass-wool. The electric furnace, L, is contained in a 10 × 20 cm. Pyrex electrolytic beaker, K, the heating unit consisting of 18 ft. of 22-gauge Chromel-A wire (1·0 ohm per foot) wound on a Transite frame, and energised by a variable transformer. Slight modifications are necessary to render the procedure applicable to fluid samples. A sample of 20 to 80 mg. selenium content is workable depending on the thiosulphate normality, size of aliquots, etc., and is added at A, either as a pellet, or weighed directly into a small platinum boat, and shaken to B. Hydrogen is passed through D at 200 ml. per min., and is ignited at C after expulsion of the original air. The oxygen supply is increased slowly to 100 ml. per min., thus giving a stable flame. The furnace and chimney assemblies are brought into place, and a current of 1 litre of air per min. through the chimney is produced by applying suction at J. Gauges are recommended for the three lines, but are not essential. The heater current is started, and raised to give a suitable rate of volatilisation of the sample as shown by the blue coloration of the otherwise colourless flame, which forms also an indicator of completeness of combustion. Most of the dioxide is deposited at F, but a significant quantity is drawn into H. In compounds where decomposition accompanies volatilisation, the dark non-volatile residue is slowly burned in the oxygen stream by gradually raising the temperature, the thermometer having been removed; the decomposition may often be avoided by using a higher temperature, or higher rate of oxygen flow. Combustion is completed in 15 to 45 min., and the furnace is lowered to permit cooling, during which time suction is maintained. The deposit is rinsed from the chimney into a 250- or 500-ml. volumetric flask; H is rinsed by passing four 25-ml. portions of water slowly up and down the glass wool, suction being applied alternately at G and J, and the rinsings added to the flask. Completeness of rinsing may be tested by adding the final water-rinsing to an acid solution of starch and potassium iodide, which should give no coloration. After filling the flask to the graduation mark, aliquot portions are titrated.



Titration of selenious acid—The dissolved oxygen is expelled by gently boiling the solution, which is then ice-cooled, and kept air-free by the periodic addition of dry ice, or by the passage of carbon dioxide, or nitrogen, through the solution. These precautions are not essential, but give slightly more accurate results. Fifteen ml. of 2 per cent. starch solution, 10 ml. of 1·5 *M* potassium iodide, and 10 ml. of 6 *N* sulphuric acid are added in that order, with thorough mixing after each addition. The solution is then titrated at once with 0·05 *N* thiosulphate until the turbid brown solution turns clear red. 1 ml. of 0·05 *N* $Na_2S_2O_3 \equiv$ 0·9870 mg. of Se.

Results and discussion—The accuracy was tested on nine, carefully purified substances containing selenium with carbon and hydrogen, and oxygen, or nitrogen, or chlorine. Experimental and theoretical results are compared, and, for the samples analysed, the maximum deviation of 1 part in 204 occurred with *p*-nitrophenolselenocyanate $C_7H_4N_2O_2Se$ of theoretical selenium content 34·77 per cent.

Volumetric methods—Compounds containing selenium-halogen or selenium-oxygen bonds may be determined iodimetrically, since they react with potassium iodide solution to give the tri-iodide. The titration of diarylselenium dihalides has been previously described (J. D. McCullough, *J. Amer. Chem. Soc.*, 1942, **64**, 2672).

Procedure for compounds reducible by potassium iodide—0·1 to 0·5 g. of the sample is weighed into 5 ml. of carbon tetrachloride, 25 ml. of 0·3 *M* potassium iodide, and 2 ml. of 6 *M* sulphuric acid and, after thorough shaking, is titrated with standard thiosulphate in the presence of starch, added near the end-point. Values are given for eleven compounds in which the maximum discrepancy is 1 part in 262 for 4-bromodiphenylselenium dibromide, $C_{12}H_9BrSeBr_2$, of theoretical equivalent 236.

Procedure for aryl diselenides—These may be titrated iodimetrically to the iodine monochloride end-point if the concentration of hydrochloric acid is kept at 5·0° to 5·5° F. at the end-point. Errors of 0·5 to 1·0 per cent. are incurred by side reactions, but the complex reactions may be more simply represented by the equations:—

$$R_2Se_2 + 6ICl \rightarrow 2RSeCl_3 + 3I_2, \text{ and}$$

$$2I_2 + IO'_3 + 6H^{\cdot} + 5Cl' \rightarrow 3H_2O + 5ICl$$

The iodine monochloride solution is prepared by titrating 1 ml. of 1·5 *M* potassium iodide in 50 ml. of 12 *M* hydrochloric acid, using 5 ml. of carbon tetrachloride for indicator purposes. When the carbon tetrachloride is just colourless, 25 ml. of 12 *N* hydrochloric acid and the weighed diselenide are added and, after shaking, the mixture is titrated with standard potassium iodate. M. E. D.

Determination of Chlorine in 2 : 2′-Dihydroxy-5 : 5′-dichlorodiphenylmethane. D. M. Jenkins, K. L. Waters, and G. D. Beal (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 609–610)—A rapid method for control purposes has been devised for the determination of chlorine in 2 : 2′-dihydroxy-5 : 5′-dichlorodiphenylmethane (DDM or Compound G–4) depending upon oxidation of the compound with alkaline potassium permanganate, acidification, and reduction of the excess of permanganate by sodium nitrite, and the determination of the liberated chloride by the Volhard method, or by photometric measurement of the turbidity of silver chloride suspensions. General applicability of the method to organic compounds containing chlorine is not claimed, but it gives satisfactory results with DDM.

Procedure—Dissolve about 0·2 g. of DDM powder in 15 ml. of 10 per cent. sodium hydroxide solution in a 500-ml. Erlenmeyer flask, add 35 ml. of saturated potassium permanganate solution, or as much more as is necessary to maintain the purple colour of the permanganate. After introducing a few glass beads, boil the mixture gently for 10 min. Cool to room temperature, add 75 ml. of water and 10 ml. of concentrated nitric acid. Reduce the excess of potassium permanganate immediately by means of 10 per cent. sodium nitrite solution, added preferably from a burette, add 25 ml. of 0·1 *N* silver nitrate, and titrate the excess with 0·1 *N* ammonium thiocyanate, 2 ml. of ferric alum solution being used as indicator. Make a blank determination with the reagents.

The analysis of emulsions containing DDM, frequently used to impart water-repellency as well as mildew-proofness to fabrics, requires little change in the procedure described. The wax used in these emulsions readily separates on the surface of the liquid during oxidation, and does not interfere with the final titration.

Owing to the small amount of DDM applied to fabrics, the chloride content of the oxidised extract cannot be conveniently titrated, but is readily determined by photometric methods, the acidity of the solution after addition of sodium nitrite being adjusted so that the silver chloride is maintained in suspension. Extract 2 g. of the very finely cut fabric in a 150-ml. beaker three times with successive 50-ml. portions of 0·025 per cent. sodium carbonate solution by boiling gently for 5 min., and decant the hot extracts into a 200-ml. flask. Wash the extracted fabric twice with 15-ml. portions of the sodium carbonate solution, and dilute the combined extracts and washings to 200 ml. with sodium carbonate solution. Filter about 50 ml. and follow the oxidation procedure already described with these exceptions. Use a 25-ml. aliquot of the filtrate, reduce the volume of potassium permanganate solution to 25 ml., and omit the dilution with water after oxidation. After reduction of the excess of permanganate by means of acid and sodium nitrite, add a few drops of phenolphthalein indicator, and then enough 10 per cent. sodium hydroxide to produce a faint pink colour. Acidify with 10 ml. of diluted nitric acid (1 + 3), transfer the liquid to a 200-ml. flask, cool to room temperature, add 4 ml. of 0·1 *N* silver nitrate, dilute to the mark with water, and invert the flask several times. After 10 min., measure the turbidity with a suitable electrophotometer (the Fisher instrument was used in the investigation), using a 525 filter, and determine the DDM content by reference to a standard curve. It is necessary to establish two "blank" values, *viz.*, an extract blank and a reagent blank. For the extract blank, pipette a 25-ml. aliquot of the filtrate into a 200-ml. flask, add 125 ml. of the sodium carbonate solution, 10 ml. of diluted nitric acid (1 + 3), and 4 ml. of 0·1 *N* silver nitrate, and dilute to the mark. Invert the flask several times and read after 10 min. This extract blank representing inorganic chlorides is often negligible. For the reagent blank, follow the same procedure, omitting the 25-ml. aliquot of the extract.

Since the logarithm of the transmittance of silver chloride suspensions is an inverse and very nearly linear function of the turbidity, four determinations are adequate to establish the standard calibration curve. Establish the curve by first dissolving 500 mg. of DDM in enough 2·5 per cent. sodium hydroxide solution to make 100 ml. of solution. Use suitable aliquots of this solution, containing 5 mg. of DDM per ml., to cover the range of 2 to 15 mg. The procedure is then as described for the fabric extract. A. O. J.

Solubility of Acetylene in Acetone. V. E. Brameld and M. T. Clark (*J. Soc. Chem. Ind.*, 1946, **65**, 58–61)—A gravimetric method for determining the solubility of acetylene in acetone has been developed, and values have been determined at 5° intervals over the range 0° to 40° C. The method is to saturate air-free acetone of B.S.509 quality with acetylene until the whole system is in equilibrium under a total pressure above the solution equal to one atmosphere plus the saturation vapour pressure of acetone at the test temperature. Thus, the solution is in equilibrium with acetylene at a partial pressure of one atmosphere. From the weights of vessel empty, plus acetone, and plus acetone and gas, the weight of acetylene dissolved by unit weight of acetone at the test temperature is calculated. Certain corrections for the air inevitably present in the acetylene are applied. Results obtained for the solubility coefficient, S_w, are:

Temp., °C.	S_w, mg. of C_2H_2/g. of acetone
0	68·7
5	61·0
10	53·5
15	46·4
20	39·4
25	32·7
30	30·0
35	25·7
40	24·4

The equation $S_w = \frac{13100}{(T-185\cdot3)} - 81\cdot3$, where T is the absolute temperature, holds over the temperature range −30° to 35° C.

The following values were found for the solubility at different partial pressures of acetylene; $S_w = S'_w/P_{Ac}$ where S'_w is the solubility under an acetylene partial pressure of P_{Ac} atmospheres.

Test temperature 30° C.

Acetylene partial pressure, atm.	S'_w, mg./g.
0·742	22·6
0·760	23·2
0·805	24·4
0·835	25·0
0·897	26·5
0·905	27·2
0·982	29·2
0·999	29·7
1·047	31·0
1·054	31·2
1·164	34·0

E. M. P.

Analysis of Dilute Melamine Resin Solutions. B. Ivarsson and B. Steenberg (*Svensk Papperstdn.*, 1946, **49**, 1; through *Tech. Bull.*, 1946, **23**, 12)—For routine work, a known wt. of the sample, as supplied for use in paper, is diluted to 35 ml., 0·1 *N* sodium hydroxide is added, and after vigorous agitation, the resulting turbidity is evaluated in a Pulfrich photometer. The method must be calibrated with known weights of resin, under constant conditions, of which the most important are the volume of sodium hydroxide added, the time elapsing after its addition, the concentration and age of the original and diluted solutions, and the presence of foreign substances, *e.g.*, salts, starch, and proteins. Methylol melamine allows of no reliable colour reactions or, because of the stability of the triazole ring, Kjeldahl determination, the picrate is too soluble to be used for a gravimetric determination, and precipitates with salts and alkali are unmanageable gels.

J. G.

Inorganic

Analysis of Boron Trifluoride in Organic Liquids (Ethers). S. L. Walters and R. R. Miller (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 658)—Hydrolysis of boron trifluoride gives a mixture of boric, hydrofluoric, and fluoboric acids, rendering determination difficult. Several methods of decomposition previously reported include fusion in a Parr sulphur bomb, attack by fuming nitric acid in a closed tube, or attack by sodium and liquid ammonia. The method described below is based on the reaction $NaF + BF_3 \rightarrow NaBF_4$. Sodium borofluoride shows no appreciable decomposition at 200° to 300° C., and can be used with solvents of relatively high boiling point.

Procedure—A stoppered, 100-ml. wide-mouthed extraction flask is weighed with 5 g. of anhydrous sodium fluoride in it. About 2 g. of sample are weighed in a tared weighing bottle (15 × 40 mm.), transfer of the sample being conveniently effected by means of a small hypodermic syringe since the compounds are hygroscopic. The weighing bottle and sample are placed in the flask, the stopper is removed, and a reflux condenser attached. After 30 minutes' heating under reflux, the liquid is evaporated on a hot-plate and the flask stoppered, cooled, and weighed. Hence the weight of boron trifluoride in the sample is given by the gain in weight less the tare of the weighing bottle. The method is trustworthy and accurate to within ±0·5 per cent. for boron trifluoride in diethyl ether, or in mixed butyl and ethyl ethers.

M. E. D.

Agricultural

Effect of Acetic Acid on the Spectrophotometric Estimation of Gossypol in Aqueous Alcoholic Solution. F. H. Smith (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 658)—Since the publication of an improved rapid method for determination of gossypol (*Ibid.*, 1946, **18**, 43; ANALYST, 1946, **71**, 339), it has been found that with different samples of alcohol the intensity of the colour formed and the precision of the analysis depend upon the acidity of the alcohol used. Very satisfactory results are obtained when sufficient acetic acid (0·1 to 0·2 ml. per litre) is present to give the 95 per cent. alcohol an acidity of 0·002 to 0·004 *N*. The upper limit of acidity is not critical, since as much as 6 ml. of glacial acetic acid per litre may be used. It is recommended, therefore, that in the determination of gossypol by the method published (*loc. cit.*) the alcohol used for the extraction be adjusted with acetic acid to an acidity between 0·002 and 0·004 *N*.

A. O. J.

Review

ORGANIC PREPARATIONS. A Translation of Part II, "Reaktionen," of Conrad Weygand's *Organisch-Chemische Experimentierkunst* (Leipzig, 1938). New York: Interscience Publishers, Inc., 1945. Price $6.00.

There have been few books of the kind which provide constant assistance in the solution of the varied problems of preparative organic chemistry. Mostly their value has been so indisputable that their use has become traditional despite the disadvantage for many chemists of their having been written in the German language. The present volume provides a translation of one of the shorter and more recent works of this kind.

The arrangement is a simple, familiar and generally satisfactory one in which the formation and fission of C–H, C–halogen, C–O and other bonds are considered in turn under immediately recognisable headings. Any uncertainty in use is banished by a subject index containing about 3,500 entries. The discussion of each topic comprises a brief commentary on general principles, and a variety of specific reactions which are frequently further illustrated by one or more examples given with experimental detail. There are a few imperfections in the text (*e.g.*, the reaction scheme on p. 176, the formula for α-ionone on p. 473, and an apparently erroneous reference on p. 96 to p. 489), but nevertheless the factual information is presented in an unusually reliable manner. It is inevitable that a text completed in 1938 should appear somewhat deficient after eight crowded years of progress in preparative organic chemistry, and this defect remains despite the insertion of a number of references to *Organic Syntheses.* It is perhaps a blemish of greater moment that although the 500 pages of this work are packed with agreeably presented facts, the information is not always what is expected in a laboratory handbook; nor is the anticipated instruction, even having regard to the date of the original compilation, always to be found within these covers. Thus, some of the brief discussions such as that on the tautomerism of nitrogen compounds (p. 489) are surely too superficial to be of much use from either the theoretical or practical standpoint. In this connection certain items strikingly lack practical application; an instance is the passing mention of the influence of glycolanilide on the saponification of esters on p. 194. In other instances the paucity of experimental material which it would have been easy to provide and which is in rather frequent demand occasions surprise. For example, the Fischer indole synthesis enjoys one paragraph without a single detailed example, and the important quinoline synthesis of Scraup (!) and the pyrrole synthesis of Knorr are dismissed in exactly those words (p. 407), though admittedly there are some references to the latter method on p. 225. Again, to take a few examples at random, there appear to be no references to such classical topics as the preparation of cyanines, thiazoles, pyrazolones or pyridines.

This work is unquestionably to be recommended as a useful and indeed substantial addition to the chemist's library, but there will be a considerable number of workers who will continue to seek assistance, even on matters which are not remote from ordinary practice, outside its pages and will look forward to the remedying of such deficiencies in future editions.

A. H. COOK.

ADVICE TO AUTHORS

THE Council has approved the following notice by the Publication Committee, which is here given in condensed form.

The Society publishes papers concerned with all aspects of analytical chemistry, inorganic and organic, including chemical, physical, biological, bacteriological and micro methods. Papers on these and allied subjects, by members of the Society or non-members, may be submitted for presentation and publication; they may:

(1) record proposals for new methods and the investigations on which the proposals are based;
(2) record the results of original investigations into known methods or improvements therein;
(3) record analytical results obtained by known methods where these results, by virtue of special circumstances, such as their range or the novelty of the materials examined, make a useful addition to analytical information;
(4) record the application of new apparatus and new devices in analytical technique and the interpretation of results.

Communications.—Papers (which should be sent to the Editor) will normally be submitted to at least one referee, on whose advice the Publication Committee will be guided as to the acceptance or rejection of any communication. Papers or Notes accepted by the Publication Committee may not be published elsewhere except by permission of the Committee.

Abstracts.—The MS. should be accompanied by a brief abstract of about 100 to 150 words indicating the scope and results of the investigation.

Proofs.—Proofs should be carefully checked and returned within 48 hours. Two galley proofs* will normally be sent out, one of which should be retained by the Author.

Reprints.—Ten Reprints are supplied gratis to the Author. Additional reprints may be obtained at cost if the Author orders them directly from the printers, W. Heffer & Sons Ltd., 104, Hills Road, Cambridge, at the time of publication. Details are sent to Authors with the proofs.

Notes on the writing of papers for THE ANALYST

Manuscript.—Papers and Notes should preferably be typewritten.

The title should be descriptive and should set out clearly the scope of the paper. Degrees are now omitted after the names of Authors in the headings of papers.

Conciseness of expression should be aimed at; clarity is facilitated by the adoption of a logical order of presentation, with the insertion of suitable paragraph or sectional headings.

Generally, the best order of presentation is as indicated below:

(*a*) General, including historical, introduction.
(*b*) Statement of object of investigation.
(*c*) Description of methods used. Working details of methods are usually most concisely and clearly given in the imperative mood, and should be given in this form, at least while economy of paper is pressing, *e.g.*, "Dissolve 1 g. in 10 ml. of water and add . . ." Well-known procedures must not be described in detail.
(*d*) Presentation of results.
(*e*) Discussion of results.
(*f*) Conclusions.

followed by a short summary (100 to 250 words) of the whole paper: items (*e*) and (*f*) can often be combined.

Illustrations, diagrams, etc.—The cost of setting up tabular matter is high and columns should therefore be as few as possible. Column headings should be brief or replaced by a number or letter to be used in combination with an explanatory footnote to the table.

Sketches or diagrams should be on white Bristol board, not larger than foolscap size, in Indian ink. Lettering should be in light pencil.

Tables or graphs may be used, but normally not both for the same set of results. Graphs should have the co-ordinate lines clearly drawn in black ink.

References.—References should be numbered serially in the text and collected in that order under "REFERENCES" at the end of the paper. They should be given in the following form:

1. Dunn, J. T., and Bloxam, H. C. L., *J. Soc. Chem. Ind.*, 1933, **52**, 189T.
2. Allen, A. H., "*Commercial Organic Analysis*," Churchill, London, 1882.

Notes on the Presentation of Papers before Meetings of the Society are appended to the "ADVICE," copies of which may be obtained on application to the Secretary, 7/8, Idol Lane, London, E.C.3.

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* During the paper shortage two copies of the MS. will not be insisted on, nor will two galley proofs be sent.

FEBRUARY, 1947 Vol. 72, No. 851

THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

PHYSICAL METHODS GROUP

The Second Annual General Meeting of the Group was held at 6 p.m. on Tuesday, November 26th, 1946, in the rooms of the Chemical Society, Burlington House, London, W.1. The Group Chairman, Mr. R. C. Chirnside, presided, and 32 members were present. The Committee's Report and the Financial Statement were presented and adopted.

The Officers and Members of Committee for the forthcoming year are as follows. *Chairman*, Mr. R. C. Chirnside. *Vice-Chairman*, Dr. J. G. A. Griffiths. *Honorary Secretary*, Dr. J. E. Page.* *Committee*, Mr. B. S. Cooper, Dr. J. R. Edisbury, Mr. J. Haslam, Dr. S. Judd Lewis, Mr. D. M. Smith, Dr. F. Wokes and, *ex-officio*, the President, Honorary Treasurer and Honorary Secretary of the Society and the Editor of The Analyst. Mr. C. A. Bassett and Dr. D. C. Garrett were reappointed Honorary Auditors.

Polarographic Discussion Panel

The Chairman said that the Group Committee had decided to form a Polarographic Discussion Panel, the objects of which would be to hold and sponsor informal discussions on polarographic analysis. Draft rules of the panel had been approved by the Committee of the Group and the Council of the Society. The following members were elected to serve on the Committee of the Panel: Dr. W. Cule Davies, Mr. J. Haslam and Mr. J. T. Stock† (Honorary Secretary), with Dr. J. E. Page as the representative of the Group Committee.

Ordinary Meeting

Immediately following the Annual General Meeting, an Ordinary Meeting of the Group was held, at which about eighty members and visitors were present. The following papers on the subject of Polarographic Analysis were presented and discussed: "Amperometric Titrations," by J. T. Stock, M.Sc., F.R.I.C.; "The Rotating Platinum Electrode," by C. J. O. R. Morris, B.Sc., Ph.D.; "The Application of the Cathode Ray Oscillograph to Polarography," by J. E. B. Randles, M.A., B.Sc., and L. Airey, B.Sc., who demonstrated their apparatus.

SCOTTISH SECTION

A meeting of the Scottish Section was held at the N.B. Station Hotel, Glasgow, on Friday, November 29th, 1946. Mr. James A. Hunter presented a paper on "A Semimicro-Method for the Determination of Magnesium," which he hopes later to submit for publication in The Analyst. Dr. C. W. Herd, B.Sc., F.R.I.C., presented "Some Observations on the new Ice Cream Order" as a basis for discussion on the subject of the Order.

DEATHS

We regret to record the death of

Joseph John Blackie
Robert Selby Morrell

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* Address: Glaxo Laboratories Ltd., Greenford, Middlesex.

† Address: Chemistry Department, L.C.C. Norwood Technical Institute, Knight's Hill, London, S.E.27.

Linlithgow Library.

Obituary

PROFESSOR W. H. ROBERTS

WILLIAM HENRY ROBERTS was born in Liverpool on January 11th, 1877. He was educated at Liverpool College and University College, Liverpool, where he studied under Professor Campbell Brown. Having taken his B.Sc. degree at Victoria University he proceeded to the M.Sc. at Victoria and Liverpool University, and in 1902 he passed the examination for the Associateship of the Institute of Chemistry.

On leaving the University, Roberts became assistant to Campbell Brown, who was not only Professor of Chemistry but also Public Analyst to the County of Lancaster.

On the death of Campbell Brown the County appointment was given to Mr. Collingwood Williams—still with us—who had held the Liverpool City Analyst's post and, as the City was contemplating the building of new City Laboratories, considerable discussion took place as to who was able to undertake satisfactorily the duties of City Analyst to Liverpool. Eventually, despite expressed doubts, Roberts was chosen and he was given as his Deputy the late Mr. E. Gabriel Jones.

Thus on January 1st, 1912, Roberts entered upon his real life's work. One of his first tasks was to plan and equip the new laboratories, which were, and are, the admiration of everyone privileged to inspect them. Those who had doubted the wisdom of the appointment were speedily converted when they saw the amazing administrative ability that the new Analyst possessed.

Roberts soon gathered round him a loyal band of able young chemists, by whom he was affectionately known as "The Chief," an appellation that later generations sometimes expanded to "The Great White Chief." The laboratories were in a new building which housed also the School of Hygiene and the City Bacteriological Laboratories, and qualified medical men reading for the D.P.H. diploma attended lectures there. It was almost inevitable that Roberts should be asked to lecture on Public Health Chemistry, and the University regularised the appointment by making him an Associate Professor.

He was allowed to use the laboratories for Public Analyst work for other towns and boroughs and held the appointments of Public Analyst for the County Boroughs of Barrow-in-Furness, Birkenhead, Blackburn, Bootle, Preston and Southport and the Boroughs of Crosby, Kendal and Widnes. He was also permitted to undertake analytical and consultative work for private firms and individuals, and the assistance that he gave to commercial enterprises still further enhanced his influence and gained a desirable publicity for the laboratories.

In 1917 he served on the Council of the Society and again in 1927 and 1936. In 1934 he was made a Vice-President and in 1938 President. This was the proudest moment in Roberts's life. The writer saw him just after he received the news and can record that this recognition by fellow workers of work well done was, to him, the highest honour which could be conferred on any chemist.

From the time he passed the examination of the Institute of Chemistry, Roberts was one of the Institute's stoutest supporters and fought for the highest standards of professional conduct. Even at the time of his retirement he was planning an attack on what he considered a reflection on the integrity of the profession. From the year 1915, with one break, his name appeared in the list of the Institute's Council, and from 1933 to 1936 and again from 1944 to 1946 he was a Vice-President; from 1925 to 1929 he acted as the Institute's examiner in Branch E (Food and Drugs). He was for many years Treasurer to the Liverpool and North-Western Section, at whose meetings he was almost invariably present and which he served zealously, being Chairman in 1921.

His interest in the Society of Chemical Industry dated back to the time he served under Campbell Brown, but naturally his influence was chiefly expended on the Liverpool Section. Even when his name did not appear on the official lists as member of Committee or Chairman of the Section, his private rooms were always at its disposal and he afforded every facility to the officers of the Section. His advice on matters of policy was greatly valued, and he was looked upon as the Father of the Section.

He encouraged any young chemist who wished to start in practice and gave every possible assistance, even allowing him to work in the Laboratory to familiarise himself with methods and apparatus, but always with the proviso that the newcomer should never attempt to cut fees or otherwise degrade or disgrace the Profession.

It would be impossible to enumerate his many benefactions, for he was the soul of generosity, and, apart from his former assistants who now hold responsible positions as Public Analysts, and managers and directors of business undertakings, hundreds of chemists have been helped and inspired by him.

He was at his best in the witness-box, and it is not too much to say that he revelled in this part of his duties.

He was a lover of humanity who tried, vainly, to hide his sympathies behind a mask of austerity. Hence he was popular with all sorts and conditions of men, from judges to criminals and from leading medical specialists to laboratory boys. Criminals have even been known to tell him a joke after he had given damning evidence against them. Socially he was what is known as a "success," but this was, to him, merely much-needed relaxation and no more His one interest in life was his profession.

He was due to retire, under an age limit clause, in January, 1942, but the Corporation felt that his services were too valuable to lose and requested him not only to retain his post but to add to his duties those of supervision of all the preparations for war-gas identification, gas damage, other damage by enemy action, water and food preservation, and so forth, "for the duration."

The death of Mrs. Roberts, a gracious and gifted lady, whose appearances at the summer meetings of the North of England Section were hailed with delight because of the atmosphere of sociability that she unconsciously diffused, and who "mothered" every member of the laboratory staff, affected Roberts profoundly. She had been his helpmeet and counsellor, restraining his impetuosity and encouraging him in his more ambitious schemes, and, with her passing, in 1942, something in Roberts died. Though he bravely tried to carry on his work as usual, those nearest to him realised how difficult he found it and how heavy was the strain under which he laboured.

He felt himself failing physically, and, from a chance remark, the writer is under the impression that he wished to "die in harness." This he nearly achieved, for during his brief period of retirement he was involved in several cases, some of which are still pending, but which he handed over to chosen confreres.

Among the large concourse that attended his funeral were representatives of the University, the City Constabulary, the Health Department and other departments of the Corporation, the legal profession, his Masonic Lodge, the Royal Institute of Chemistry, the Chemical Society, the Society of Chemical Industry, and other local Societies. The staff of the City Laboratories and a number of Public Analysts from the North also attended.

Mr. S. E. Melling (Past President) officially represented the Society.

F. ROBERTSON DODD

Some Remarks on the Statistical Background in Bio-Assay

By E. C. FIELLER

(Read at the Annual General Meeting of the Biological Methods Group, February 25th, 1946)

1. In properly conducted biological assays (and this is now well recognised) the experimenter has a dual aim—firstly, to estimate the activity of a test preparation in terms of that of a standard, and secondly, to derive an objective measure of the reliability of his estimate. The task of evolving an assay procedure is a joint one for the biochemist and the statistician, but it is unwise, as well as increasingly difficult, to attempt any precise definition of their respective fields; they can collaborate effectively only through mutual adaptation. Broadly speaking, however, it is impossible to conceive of a rational assay-procedure for which there is an experimental technique without an accompanying arithmetical technique, and it is with the latter that I shall be concerned.

2. Like Mr. Gridgeman,[12] I shall restrict myself mainly to the last of the three assay-types that he distinguishes, that in which the response to any particular dose is regarded as a continuous variate whose mean value is linearly related, throughout the effective dosage-range, to the logarithm of the dose. This assumption of linearity is not in itself sufficient to provide a basis for deriving, by a rigid mathematical argument, the formulae by which such assays are usually interpreted. We need two further assumptions, that the distributions of responses about their mean values follow the normal law of errors, and that the variances of these distributions are, over the effective dosage-range, equal. If these three conditions

of linearity, normality and stability of variance are assumed to be satisfied, the design and interpretation of an assay become relatively simple. The safest procedure is to have experimental groups on at least three different dose-levels both of the standard and of the test preparation. The assay then becomes self-contained: in order to interpret it we need not appeal to previous experience for estimates of the log (dose) - response line* or of the variance of individual responses, and we can, moreover, check what for the time being I shall take for granted—the adequacy of the assumptions that we have made.

3. Let us suppose now that in an assay in which the experimental groups have been chosen at random from the laboratory stock, the doses of standard (measured on a logarithmic scale), the numbers of individuals responding to them, and their average responses are respectively

$$\begin{array}{cccc} x_{11} & x_{12} & x_{13} & \cdots\cdots \\ n_{11} & n_{12} & n_{13} & \cdots\cdots \\ y_{11} & y_{12} & y_{13} & \cdots\cdots \end{array}$$

while the corresponding quantities for the test preparation are

$$\begin{array}{cccc} x_{21} & x_{22} & x_{23} & \cdots\cdots \\ n_{21} & n_{22} & n_{23} & \cdots\cdots \\ y_{21} & y_{22} & y_{23} & \cdots\cdots \end{array}$$

If we write

$$\begin{array}{ll} n_1 = n_{11} + n_{12} + n_{13} + \cdots\cdots & = \Sigma n_{1i}, \\ x_1 = \Sigma n_{1i} x_{1i}/n_1, & y_1 = \Sigma n_{1i} y_{1i}/n_1, \\ p_1 = \Sigma n_{1i}(x_{1i} - x_1)^2, & q_1 = \Sigma n_{1i}(x_{1i} - x_1)(y_{1i} - y_1), \end{array}$$

with similar meanings for n_2, x_2, y_2, p_2, q_2, and finally

$$p = p_1 + p_2, \qquad q = q_1 + q_2,$$

then we estimate β, the slope of the log (dose) - response line, as $b = q/p$, and the activity of the test preparation from the familiar formula[11,13]

$$\log \frac{\text{activity of test}}{\text{activity of standard}} \simeq \mathrm{L} = (x_1 - x_2) - \frac{y_1 - y_2}{b} = \mathrm{X} - \frac{\mathrm{Y}}{b}, \text{ say.} \quad \ldots \quad (1)$$

The estimate L is subject to sampling variations, since both the numerator and the denominator of the fraction Y/b are; if the protocols of the assay supply an estimate s^2 of the common variance σ^2 of individual responses, then in assays of the unrestricted design under consideration Y and b are distributed normally and independently with variances that we can estimate as

$$\mathrm{V(Y)} = s^2\left(\frac{1}{n_1} + \frac{1}{n_2}\right), \qquad \mathrm{V}(b) = \frac{s^2}{p}.$$

Suppose now that t is, say, the 5 per cent. point of the Student-distribution[10] with the same number of degrees of freedom as s^2. Then as long as $[b^2 - t^2\mathrm{V}(b)]$ is positive, the 95 per cent. fiducial limits for $\lambda - \mathrm{X}$, where λ is the true logarithm of the activity ratio, are given by the quadratic equation (6,7,9)

$$\lambda^2[b^2 - t^2\mathrm{V}(b)] + 2\lambda b\mathrm{Y} + [\mathrm{Y}^2 - t^2\mathrm{V(Y)}] = 0 \quad \ldots \quad \ldots \quad \ldots \quad (2)$$

It follows easily, on solving this equation, that if

$$\mathrm{C} = b^2/[b^2 - t^2\mathrm{V}(b)],$$

$$\mathrm{L}' = \mathrm{X} - \mathrm{CY}/b$$

and

$$s_{\mathrm{L}}'^2 = \mathrm{C}\frac{s^2}{b^2}\left[\frac{1}{n_1} + \frac{1}{n_2} + \frac{\mathrm{C}}{p}\left(\frac{\mathrm{Y}}{b}\right)^2\right], \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (3)$$

then the fiducial limits for λ are $\mathrm{L}' \pm ts_{\mathrm{L}}'$. A numerical illustration is given in B.S.I. Specification No. 911[5]: the calculation of limits set out there might possibly be simplified by replacing (3) by the equivalent formula

$$t^2 s_{\mathrm{L}}'^2 = (\mathrm{C} - 1)\left[p\left(\frac{1}{n_1} + \frac{1}{n_2}\right) + \mathrm{C}\left(\frac{\mathrm{Y}}{b}\right)^2\right]$$

4. It may conveniently be remarked at this stage that although the assumption of normality is necessary for a rigid derivation of the above formulae, its failure in practice entails

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* *I.e.*, the line showing the relation between x and η, where η is the mean response in a large group of animals each receiving a dose the logarithm of which is x.

little loss. This is in keeping with the general fact, established by many practical and theoretical investigations (*e.g.*, Welch[22]), that statistical tests based on the analysis of variance remain stable even when the parent population departs quite widely from the normal form. In particular, the part played by the assumption of normality in the mathematical argument of paragraph 3 is only a minor one; it consists principally in ensuring the normality of the sampling distributions of the estimates Y and b, but since these estimates are weighted means of all the responses y observed in the assay, their distributions will in any case be effectively normal if each y has the same error-variance σ^2. Accordingly, we can regard the argument of paragraph 3 as dependent only on the assumptions of linearity and stability of variance.

5. The fiducial-limit formulae in the form given above hold whenever, as in completely balanced litter-mate assays, Y and b are uncorrelated. I have indicated elsewhere[6] two other methods by which they may be established; one of them, the geometric approach suggested by Fisher's account[9] of the work of Working and Hotelling and by Bliss,[3] is very clearly elaborated by Irwin in his well-known 1943 paper.[14] The present method is to be preferred, since it can be adapted immediately to the calculation of limits for assays in which Y and b are not estimated independently. This is, in general, the position in the twin cross-over test,[21] for example, and in litter-mate assays in which some of the "responses" have to be estimated. All that is necessary in such instances is to replace the second term in (2) by $+\ 2\lambda[b\text{Y} - t^2\text{Cov}(b\text{Y})]$.

6. In a self-contained assay carried out on an assigned number of animals (or other test organisms), in which the assumptions of linearity and stability of variance are sufficiently realistic working hypotheses, we shall achieve maximum precision by minimising $s_{\text{L}}'^2$. This entails four requirements, which can conveniently be considered in turn,—minimising $\left(\frac{1}{n_1} + \frac{1}{n_2}\right)$ and $\left(\frac{\text{Y}}{b}\right)^2$, and maximising p and b^2/s^2 (which will incidentally minimise C).

(i) It is easily seen that, if $(n_1 + n_2)$ is fixed, $\left(\frac{1}{n_1} + \frac{1}{n_2}\right)$ is least when n_1 and n_2, the numbers of responses to test and standard, are equalised. In practice, if there is any great doubt about the activity of the test preparation, it may pay to have more dosage groups on test than on standard, to allow for the possibility that the extreme ones may eventually be rejected as falling beyond the range of linearity. More generally, in a multiple assay in which k test preparations are being compared simultaneously with standard, we need to minimise $\left(\frac{1}{n_1} + \frac{1}{n_2}\right)$ subject to $(n_1 + kn_2)$ being fixed; the solution is $n_1 = n_2\sqrt{k}$.

(ii) To minimise $(\text{Y}/b)^2$, it is necessary—assuming for the moment that the slope β is fixed—to minimise Y^2, *i.e.*, to aim at equalising the average responses to test and standard.

(iii) To maximise p, we need to maximise both p_1 and p_2. If with each preparation we are willing to sacrifice the test for linearity, and can determine the extreme doses between which the log (dose) - response line is straight, it is most economical to put equal groups on these doses.

(iv) We may regard b^2/s^2 as an approximation to β^2/σ^2 ($1/\lambda^2$ in the notation of Bliss and Cattell[4]), where β and σ^2 are, as above, the true slope of the log(dose) - response line, and the true residual variance about it. Maximising β^2/σ^2 is primarily a matter for the biochemist rather than the statistician, since β^2 increases with the sensitivity of the test-organisms to changes of dose, and $1/\sigma^2$ with the uniformity of their responses; the fact that the precision of the assay depends on the ratio of β^2 to σ^2 is worth stressing—it implies that it is pointless to steepen the slope β if so doing entails a proportionate, or more than proportionate, increase in σ.

7. There is, however, one contribution that the statistician may make to the reduction of β^2/σ^2. In many assays the response of the individual animal can be shown to depend not only on the dose it receives, but also on the value of some characteristic, such as its weight or blood-sugar level, at the time it is dosed. This may well happen, in particular, when the metameter is obtained by comparing the final value of the characteristic with its initial value, and is provisionally calculated by the arbitrary device of taking a difference or a percentage. In such instances, the disturbing effect of differences in the initial values can, as Gridgeman has mentioned,[12] be estimated and removed by Fisher's technique of covariance analysis, which produces a modified metameter, uncorrelated with the initial readings, for which σ^2 is reduced but β^2 unaltered. Many examples will be found in the work of Bliss and

his associates. In this connection one computational point may be mentioned, since it is frequently overlooked. If we estimate the covariance correction from the internal data of an assay, the logarithm of the activity ratio is estimated not as in the second member of (1), but as

$$\mathrm{X} - (\mathrm{Y}'/b') = \mathrm{X} - (\mathrm{Y} - h\mathrm{Z})/(b_y - hb_z), \quad .. \quad .. \quad .. \quad (5)$$

where h is the estimated residual regression of the responses y on the initial readings z, and Z and b_z are calculated from the recorded values of z by the same steps that produce Y and b_y from the recorded values of y. This method of allowing for initial differences thus introduces a covariance $\mathrm{Z}b_z\mathrm{V}(h)$ between Y′ and b', and to calculate fiducial limits correctly we must modify the quadratic equation (2) in the way described in paragraph 4. The necessity for this modification vanishes, of course, if we can estimate h from previous experience, as proves possible in the assay of insulin by the rabbit method.[8,21]

8. The ratio β^2/σ^2 gives the best indication of the reliability of an assay method of the type we are considering, because it is inversely proportional to the number of animals needed, on the average, to give any assigned degree of accuracy. If a laboratory is repeatedly carrying out routine assays by the same technique, the estimate b^2/s^2 is the most appropriate single quantity to watch in a control chart, although it may be profitable to run subsidiary charts of b^2 and s^2 as well.[17] If rival assay-methods are available for the same purpose, as are the line-test and the bone-ash test for vitamin D_2, and if the reliability of each method is substantially constant, then the respective values of β^2/σ^2, divided by the cost per animal, provide an appropriate criterion for judging between the methods. If their reliability may change from time to time (which is what we assume may happen, when we insist that each assay should be self-contained) then we must judge between the rival methods by judging between the respective distributions of β^2/σ^2, divided by the cost per animal. In this latter case it is open to debate whether we should decide solely by the mean values of the criteria, and if not, how far we should be influenced by their stability about their mean values.

9. The considerations of paragraph 8 can profitably be extended to any assay in which the response is a continuous variate. Let us suppose that doses are still measured on a logarithmic scale, but that the mean response to any particular dose x is now a non-linear function $\bar{y}(x)$ of x, and the variance of individual responses another function $\mathrm{V}\{y(x)\}$ of x. In place of the slope β of the straight log (dose) - response line, it is natural to consider the gradient $\frac{d}{dx}\bar{y}(x)$ of the log (dose) - response curve, and in place of the single criterion of reliability β^2/σ^2 ($= \alpha$, say), we can consider the *local* criterion of reliability[*]

$$\alpha(x) = \left\{\frac{d}{dx}\bar{y}(x)\right\}^2 \div \mathrm{V}\{y(x)\} \quad .. \quad .. \quad .. \quad (6)$$

which will in general vary with x. If we knew $\alpha(x)$ precisely, we should choose, as the most economical one in which to carry out assays, the region of x in which $\alpha(x)$ is greatest; if the conditions of linearity and stability of variance are exactly satisfied over some range of x, then $\alpha(x)$ must be constant over that range. To the first order of approximation at any rate—and this is in keeping with the last two remarks—$\alpha(x)$ is independent of the scale on which we choose to measure the response. To demonstrate this, consider the effect of the transformation

$$z = f(y) \quad .. \quad .. \quad .. \quad .. \quad .. \quad (7)$$

on the group of responses to some constant dose. If $\bar{y}$ is their mean, we can represent the typical response as $\bar{y} + (y - \bar{y})$, and its transform by

$$z = f(\bar{y}) + f'(\bar{y})(y - \bar{y}) \text{ approximately.} \quad .. \quad .. \quad .. \quad .. \quad (8)$$

As we move from one response to another within the group, $f(\bar{y})$ and its first derivative $f'(\bar{y})$ remain constant, and on the average $(y - \bar{y})$ is zero, so that

$$\bar{z} = f(\bar{y}), \text{ and } z - \bar{z} = f'(\bar{y})(y - \bar{y}),$$

whence we derive immediately the familiar result

$$\sigma_z = f'(\bar{y})\sigma_y \quad .. \quad .. \quad .. \quad .. \quad .. \quad (9)$$

---

[*] The sense in which we can regard $\alpha(x)$ as a local criterion of reliability becomes apparent if we consider the idealised case in which an assay is carried out on very large dosage groups, the successive doses of test and standard lying so close together that over their range the variations in the numerator and denominator of $\alpha(x)$ are negligible. If we interpreted the protocols of this imaginary assay by the formulae of paragraph 3, we should expect to find b^2/s^2 approximating to $\alpha(x)$.

or, in the notation of equation (6),

$$\mathrm{V}\{z(x)\} = \left\{\frac{d\bar{z}(x)}{d\bar{y}(x)}\right\}^2 \{\mathrm{V}y(x)\}.$$

Since $\dfrac{d\bar{z}(x)}{d\bar{y}(x)}\,\dfrac{d\bar{y}(x)}{dx} = \dfrac{d\bar{z}(x)}{dx}$, it follows that approximately

$$\left\{\frac{d}{dx}\bar{z}(x)\right\}^2 \div \mathrm{V}\{z(x)\} = \left\{\frac{d}{dx}\bar{y}(x)\right\}^2 \div \mathrm{V}\{y(x)\} \qquad \ldots \quad \ldots \quad (10)$$

i.e., $\alpha(x)$ is unaffected by the transformation (7).

10. The scale of measurement on which responses can conveniently be recorded in the laboratory is not necessarily the one on which the arithmetic of the assay should be performed. By transforming the response, it is frequently possible to bring within the scope of the familiar formulae of paragraph 3 an assay-procedure that at first sight seems to lie outside it. The considerations of paragraph 9 imply that if a metameter can be found that satisfies the two conditions of linearity and stability of variance, and if the recorded responses are transformed so that they fulfil one of these conditions, then they will automatically fulfil the other. Nevertheless, it seems preferable to aim primarily at stabilising the variance. I believe that usually this procedure will in fact reveal a sufficiently wide range of doses over which the curve relating responses to logarithms of doses may be taken as linear, and in which, for maximum precision, assays should be planned; even if it does not, it is not difficult to see how to allow for curvature in estimating the logarithm of the activity ratio and calculating its fiducial limits.[6] It seems likely, on the other hand, that to allow for real changes in the variance, as we move along the dosage range, would inevitably entail more complicated arithmetic, the results of which, moreover, could only be regarded as approximate.

11. The transformation required to stabilise the variance follows immediately from equation (9). Let us suppose that by plotting the standard deviation against the mean, for different dosage groups, we find them to be connected by the relation

$$\sigma_y = \phi(\bar{y}). \qquad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (11)$$

The transformation (7) will produce a metameter z for which

$$\sigma_z = f'(\bar{y})\,\phi(\bar{y}),$$

and σ_z will therefore be constant if $f'(\bar{y})\,\phi(\bar{y})$ is, so that we must take $f(y)$ proportional to

$$z = \int \frac{dy}{\phi(y)}. \qquad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (12)$$

It may be useful to draw attention, in particular, to the logarithmic and square-root transformations. The former should be applied if σ_y is proportional to $\bar{y}$, the latter if it is proportional to $\sqrt{\bar{y}}$, since

$$\int \frac{dy}{y} = \log y, \qquad \int \frac{dy}{\sqrt{y}} = 2\sqrt{y}.$$

12. As examples of the use of these two transformations, we may refer to the assay of vitamin D_3 by the radiographic technique, [19, 2] and to the assay of vitamin B_1 by the rat-bradycardia method. Olsson[20] gives details of a typical D_3 assay in which, with the tarso-metatarsal distance as metameter, the observed mean responses and estimated standard deviations within dosage-groups of 28 to 30 chicks were as follows (Table I):

TABLE I

OLSSON'S VITAMIN D_3 ASSAY DATA

(Metameter y = TMT distance)

Cod liver oil I				Cod liver oil II			
Group No.	Dose g.*	Mean $\bar{y}$	S.D. s_y	Group No.	Dose g.*	Mean $\bar{y}$	S.D. s_y
1	0·20	2·06	0·68	1	0·25	2·14	0·74
2	0·40	1·63	0·41	2	0·50	1·70	0·62
3	0·80	1·22	0·39	3	1·00	1·26	0·53
4	1·60	0·96	0·19	4	2·00	1·00	0·16
5	3·20	0·96	0·22	5	4·00	0·98	0·18

* Per kilogram of food.

In the early assays carried out in this country in 1939 and 1940 by the same method, it was observed that σ_y decreased with $\bar{y}$ in a similarly rapid manner, but that the use of the logarithmic transformation usually reduced the differences between the group variances to insignificance.[2] In B.S.I. Specification No. 911 it was accordingly recommended that

$$100 \log_{10} (10 \times \text{TMT distance in mm.})$$

should be used as metameter.[5] Jones and Elliot[16] later found, for their own assays, that in many instances the calculations of activity and of fiducial limits produced virtually identical results, whether carried out on the observed distance or on its logarithm. This does not imply that there is no need to transform the distance, since in other instances the results are not identical, and since in the laboratory in question the variance of the untransformed distance is by no means stable,[15] but it does suggest that the topic might bear re-examination.

13. The square-root transformation is of course particularly easy to handle, since the recorded responses are themselves the squares of the metameter used in the arithmetic, and it appears to apply with remarkable fidelity in the rat-bradycardia assay of vitamin B_1. Table II, based on Table II of Baker and Wright's survey[1] of assays carried out during 1937–9, shows that with the duration of cure as metameter the mean response was almost exactly proportional to the group variance. (Pooling the data is legitimate because, as has been reported elsewhere,[18] the log (dose) - response line for the laboratory in question undergoes little variation in slope or position.) The use of the square-root transformation is clearly indicated; Table III shows the extent to which it succeeded in stabilising the variance when applied to tests carried out, six years later, in the summer of 1945.

TABLE II

MEAN RESPONSES AND GROUP VARIANCES IN RAT-BRADYCARDIA ASSAYS FOR VITAMIN B_1

1937–9 Data: Metameter y = duration of cure in days

Dose mg.*	No. of responses	Mean $\bar{y}$	Variance s_y^2	Ratio s_y^2/y
15	501	3·10	1·59	0·50
25	483	4·49	2·[illegible]0	0·55
40	4[illegible]5	5·97	3·21	0·54

* Old International Standard (acid-clay adsorbate).

TABLE III

MEAN RESPONSES AND GROUP VARIANCES IN RAT-BRADYCARDIA ASSAYS FOR VITAMIN B_1

1945 Data: Metameter y = duration of cure in days

Dose μg.*	No of responses	Mean $\bar{y}$	Variance s_y^2
3	41	1·61	0·057
6	41	1·89	0·063
9	41	2·24	0·064

* New International Standard (crystalline vitamin B_1).

14. The well known result expressed by equation (12) could easily be applied (although as far as I know it never has been) without the intervention of formal mathematics. From the experimental records we could plot on a graph one point for each separate dosage group, the abscissa of the point being the mean response in the group, and the ordinate the *reciprocal* of the estimated standard deviation. A smooth curve drawn through the swarm of points thus obtained would approximate to the graph of $1/\phi(y)$, and the transformed value of any particular response y could be obtained mechanically as the area enclosed by this curve, the axes of $\bar{y}$ and s_y, and the ordinate at $\bar{y} = y$. It would thus be possible to build up empirically what we finally require for application—a table showing the values of z corresponding to selected values of y.

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I am indebted to the directors of Vitamins Limited for permission to quote paragraph 13 above from an unpublished survey of the rat-bradycardia method of assaying vitamin B_1, and, more generally, for affording me from 1942 onwards the opportunity to remain in touch with a subject that my war-time duties would otherwise have compelled me to abandon.

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A New Titrimetric Method for the Estimation of Fluorine

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INTRODUCTION—

Despite the importance attached to the distribution of fluorides in micro quantities in foods, water, etc., and the consequent effect upon teeth, it is only comparatively recently that it has been possible to estimate such traces with any degree of accuracy. The colorimetric method of De Boer,[1] based upon the fading of a zirconium alizarin lake, was used for many years as the standard micro procedure, and modifications of this are still favoured in waterworks practice. The work of Willard and Winter[2] showed the limitations of this procedure, particularly with regard to rate of fading of the lake when fluorides were added. They suggested instead a method based on titration of the fluoride solution with thorium nitrate and use of zirconium alizarin sulphonate to indicate the point when an excess of thorium over and above that required to combine with fluorine was present in the solution. This technique was improved by Armstrong,[3] who suggested a dilute alizarin sulphonate solution as the indicator, by Hoskins and Ferris,[4] by Rowley and Churchill[5] and finally by Eberz, Lamb and Lachele.[6] The technique advocated by the last-named authors is capable of giving reproducible results after some experience, but the end-point is rather vague and there is some lag in the completion of the reaction. This factor has been reduced to some extent by the back-titration technique of Dahle *et al.*,[7] and Elsworth,[8] and has been further clarified in the Society's official publication on the subject.[9]

The possibility of using other dyestuffs instead of alizarin in the formation of the thorium lake has been investigated, and we have shown that Solochrome Brilliant Blue B.S. (Colour Index 723), which is the sodium salt of sulpho-dichlorohydroxy dimethylfuchsin dicarboxylic acid, is suitable for this purpose. It possesses the following advantages over alizarin:

(1) The colour change is sharp from pink to blue, and is more sensitive than that usually given by alizarin methods.

(2) The reaction is immediate, no lag being observed.

OUTLINE OF METHOD—

Samples containing fluorides, after suitable treatment, are buffered to *p*H 3·0 and titrated with thorium nitrate in presence of Solochrome Brilliant Blue B.S. until the colour obtained matches a blank consisting of a trace of thorium nitrate added to a buffered solution of the dyestuff. The method is standardised against known amounts of fluoride and a calibration curve is prepared relating titre of thorium solution to fluorine content. The method is suitable for amounts of fluorine from 2 μg. to 100 μg. With larger quantities, thorium fluoride comes out of solution and affects the colour matching. Suitable aliquots are therefore titrated to keep within these limits. Alternatively a protective colloid may be used whereby thorium fluoride is maintained in solution and the titratable range increased accordingly.

INVESTIGATION INTO OPTIMUM CONDITIONS—

(*a*) *Volume of titrating solutions*—

Varying the amount of the final titrating solutions has been investigated to ascertain the effect of dilution upon the end-point. It is established that up to 100 ml. can be titrated as readily as 25 ml. without increasing the concentration of the indicator. Thus although the depth of colour is weaker, it is still quite readily matched. The method should, however, be standardised on a volume equal to that to be titrated in the particular determination.

TABLE I

Volume of sample solution titrated, ml...	10	25	100	10	25	100
Fluorine present, μg.	20	20	20	40	40	40
0·004 *N* Thorium nitrate required, ml. ..	0·71	0·72	0·71	1·40	1·39	1·44

(*b*) *Use of buffer*—

The use of a buffer prepared from half-neutralised chloroacetic acid (0·24 *M*) assures that the titration is carried out at *p*H 3·0 or thereabout. At this point very reproducible results are obtained. Titration in neutral solution is indefinite, and the sharpness of end-point is increased with increasing acidity until *p*H 3·0 is reached. Solutions more acid than this do not give good reproducibility on titration. The use of the acid buffer also allows of greater sensitivity—thus twice as much thorium solution is required at *p*H 3·0 (3·12 ml. per 100 μg. of F) as at *p*H 7·0 (1·25 ml. per 100 μg. of F).

(*c*) *Concentration of dye*—

The amount of dye used does not seem to affect the titration figure of a solution, but ease of matching is greatly influenced. Thus 0·5 ml. of 0·01 per cent. solution in 50 ml. of liquid gives a very pale colour the change of which is not easy to judge, and 3·0 ml. of 0·01 per cent. solution has so much basic residual red colour that the blue colour of the thorium lake is masked. In practice the aim is to obtain the sharpest colour change with the largest amount of dye, and this occurs with 2·0 ml. of a 0·01 per cent. solution, in 50 ml. volume.

(*d*) *Excess of thorium solution added*—

The end-point of the titration is assessed by comparing the tube with a similar tube to which all the reagents and a small quantity of thorium solution are added. The amount of the excess of thorium added greatly affects the titration and it is essential that the calibration curves relate to the conditions of experiment. It is not clear why the titration figure should vary with the excess of thorium added, and it would appear that the thorium fluoride itself enters into the formation of the lake. Precautions must be taken that the amount of thorium nitrate added to the comparison tube is always the same as that used when the calibration curve is prepared. Table II shows the volumes of 0·004 *N* thorium solution required for 50 μg. of fluorine corresponding to different volumes added to the blank.

TABLE II

Thorium solution added to blank ml.	Thorium solution required by 50 μg. F. ml.	Thorium solution required by 50 μg. F. corrected for blank ml.
0·05	1·55	1·50
0·08	1·62	1·54
0·10	1·84	1·74
0·15	2·09	1·94
0·20	2·36	2·16
0·30	2·78	2·48

Although higher titration figures are obtained with increased additions to the blank, if the quantity added is above 0·10 ml. the end-point tends to become difficult to recognise.

EFFECT OF SUBSTANCES OTHER THAN FLUORINE ON THE TITRATION—

The method is not specific for fluorine, as many substances that form complex ions with thorium will behave in a similar manner.

The effects of some interfering substances are recorded in Table III.

TABLE III

EFFECT OF INTERFERING SUBSTANCES ON TITRATION OF 40 μg. OF FLUORINE

Alcohol: ml. present	0	1	5	
Titration figure, ml.	1·41	1·41	0·91*	
Sodium chloride: mg. present	0	10	100	500
Titration figure, ml.	1·41	1·53	1·54	1·58*
Sodium carbonate: mg. present	0	1	3	
Titration figure, ml.	1·41	1·38	—†	
Sodium sulphate: mg. present	0	1	10	
Titration figure, ml.	1·41	1·46	1·70*	
Sodium perchlorate: mg. present	0	10	100	
Titration figure, ml.	1·41	1·43	1·43	
Sodium borate: mg. present	0	1	10	
Titration figure, ml.	1·41	1·40	—†	
Sodium acetate: mg. present	0	1	10	
Titration figure, ml.	1·41	1·39	1·08	
Sodium citrate: mg. present	0	1	2	
Titration figure, ml.	1·41	(Basic colour only)		
Potassium chloride: mg. present	0	10	100	
Titration figure, ml.	1·41	1·43	1·47	
Sodium silicate: mg. present	0	10	100	
Titration figure, ml.	1·41	1·41	1·04	
Sodium phosphate: mg. present	0	5	100	
Titration figure, ml.	1·41	—†	—†	
Sodium tartrate: mg. present	0	1	5	
Titration figure, ml.	1·41	—†	—†	

* Poor end-point. † Decolorised.

It is concluded from these experiments that the method is applicable only to relatively pure solutions of fluorides and that with few exceptions most other ions modify the titration value. This is also the experience of workers on the de Boer technique and the alizarin-thorium titration method of Willard and Winter. It is usually necessary, therefore, to separate the fluorine from such interfering substances and this is most conveniently done by distillation as silicon tetrafluoride by heating with silica and an acid of high boiling-point.

DETAILS OF DISTILLATION OF FLUORINE—

The experimental work on the effect of interfering substances indicates that distillation of the fluorine should best be carried out with perchloric acid, since traces of any acid used are certain to distil over and the salts of this acid have least effect on the subsequent titration. Other workers[6] have shown that distillation is effective with this acid, and with glass wool as a source of silica. Ellsworth[8] investigated the temperature of distillation and showed that satisfactory results are obtained if the range is kept between 135° and 145° C. An all-glass distillation apparatus is preferred. The sample, together with about 10 ml. of perchloric acid, is placed in the distillation flask. A thermometer in an elongated ground jointed mercury pocket which is of such length that it dips below the surface of the liquid. Water is added from a funnel at such a rate and the bunsen so adjusted that distillation proceeds at between 135° and 145° C. To ensure complete distillation of all the fluorine, about 200 ml. of distillate must be collected, although about 80 per cent. of the fluorine comes over with the first 50 ml. If the temperature of distillation falls below 135° C., the removal of fluoride becomes incomplete even with 200 ml. of distillate. A distillation temperature above 145° C. results in the distillation of excessive amounts of perchloric acid, which may modify the subsequent titration.

Blank distillations show that a titration figure is always obtained, equivalent to about 4 μg. of fluorine. This is said to be derived from the glass distillation apparatus. Higher blanks than this are usually due to impurity in the perchloric acid, which should be heated to 140° C. and then redistilled before use.

The type of silica used in the distillation is important. Some authors suggest powdered glass, but glass wool seems to be most satisfactory. Precipitated silica gel is definitely unsatisfactory. Some experiments in which silica gel, obtained during heating of the sample in glass vessels with caustic soda, was present, gave results corresponding to only about 40 per cent. recovery. When silica gel was added to standard fluoride solution before distillation not more than 80 per cent. recovery was obtained. Complete recovery could be obtained by using glass wool in the distillation flask.

CALIBRATION CURVE—

Although the method as outlined is capable of giving reproducible results without difficulty, it is suggested that a calibration curve correlating titration figure and fluorine content be made each time a change in the conditions is encountered. The lake formed with thorium salts and the dye is of variable composition and in consequence slight modifications in the conditions may influence the end-point.

Taking the concentrations of reagents found to be optimum and titrating with 0·004 *N* thorium nitrate solutions, a typical calibration series is as follows.

TABLE III

Fluorine present, µg.	5	10	20	40	60	80	100
0·004 *N* Thorium nitrate solution required, ml.	0·25	0·40	0·72	1·39	2·00	2·69	3·28

METHOD FINALLY ADOPTED

REAGENTS—*Perchloric acid*: 60 per cent. (pure); redistilled. *Glass wool*: fluorine-free. *Standard thorium nitrate solution*: 0·004 *N*; dissolve 552 mg. of $Th(NO_3)_4.4H_2O$ in 1 litre of water. *Solochrome Brilliant Blue B.S.*: 0·02 per cent. solution in water. *Chloroacetic buffer*: dissolve 22·7 g. of chloroacetic acid to 100 ml. in water; titrate 50 ml. of this solution with 6 *N* sodium hydroxide to neutralise; combine the two portions and dilute with water to 1 litre.

PROCEDURE—Ash the sample containing the fluoride after heating to dryness with calcium oxide according to the procedure recommended in the Society's publication.[9] Transfer the ash with the aid of a minimum quantity of water to a distilling apparatus (described above), add a small quantity of silver perchlorate to precipitate any chloride present, and allow the distillation to proceed. Place about 0·1 g. of glass wool and 15 ml. of perchloric acid in the distillation flask with the sample and add water *via* a dropping funnel continuously at such a rate that, with the heating suitably adjusted, the distillation temperature is maintained between 135° and 145° C. Distil about 200 ml. of liquid.

Dilute the distillate to a known volume, and measure an aliquot containing less than 100 µg. of fluorine for titration. Transfer the aliquot to a Nessler glass standing on a white tile, and neutralise it to phenolphthalein end-point with dilute alkali. Then just discharge the pink colour by adding a dilute solution of perchloric acid. Add 1 ml. of dye solution and then dilute perchloric acid solution until the yellow colour of the dye just changes to pink. Then add 0·5 ml. of chloroacetic buffer solution. Into a similar Nessler tube introduce a volume of distilled water equal to that of the sample, one ml. of dye solution and 0·5 ml. of chloroacetic buffer. To this tube add also 0·1 ml. of 0·004 *N* thorium nitrate solution accurately from a micro-burette. The colour changes from pink to bluish purple. Then titrate the unknown sample titrated with 0·004 *N* thorium nitrate until it exactly matches the blank in colour. From the titration figure subtract 0·1 ml. and refer the result to the calibration curve to obtain the concentration of fluorine in the sample.

Table IV shows results of some determinations carried out in triplicate on known amounts of fluorides, submitted to the distillation technique and titrated by the method described.

TABLE IV

Fluorine in fluoride taken µg.	Fluorine found µg.
1·9	1·5; 1·7; 2·1
3·8	3·7; 3·5; 4·0
7·6	7·6; 7·4; 7·4
38	37·6; 37·6; 38·2
76	75·2; 76·1; 75·8
95	95·0; 94·6; 94·4

ADAPTATION OF THE METHOD TO COLORIMETRIC TECHNIQUE—

The principle of the method described above may be applied to a colorimetric technique. In this case there is formed a blue lake with thorium nitrate and the dye. The fluorine solution is then added and the colour of the solution is diminished proportionately to the formation of the thorium fluoride complex. This reduction of colour intensity may be measured—most conveniently on the Spekker absorptiometer. The method is standardised by means of a number of standard fluoride solutions treated in the same manner. As in the titrimetric technique, rigid adherence to the conditions of calibration is necessary since the colour intensity is dependent upon the concentration of all substances in solution.

The use of the colorimetric technique is not recommended. It requires more careful control and is not capable of the same degree of accuracy.

SUMMARY—

1. A new method is given for the estimation of small amounts of fluorine. It is based upon titration of the fluoride solution with thorium nitrate solution until excess is shown by the formation of a lake with Solochrome Brilliant Blue B.S. Optimum conditions for the estimation are described.

2. The method has the following advantages over the existing alizarin technique.

(*a*) The end-point is more definite and easily judged and therefore allows of greater sensitivity;
(*b*) The end-point is immediate;
(*c*) The colour does not fade.

3. The method allows the estimation of from 2 μg. to 100 μg. of fluorine within the accuracy of titration from a micro-burette, *i.e.*, 0·02 ml., or equivalent to about 0·5 μg. of fluorine.

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The Estimation of the Volatile Matter Content of Propellant Explosives

Part 2.* The Estimation of Ethyl Alcohol and Ether

BY T. G. BONNER

IN the manufacture of nitrocellulose powders a mixed solvent of ethyl alcohol and ether is invariably used to facilitate the mixing of constituents and to ensure homogeneity of the product. The removal of these solvents in the final stage of manufacture is never complete, and a small residuum is always tenaciously retained in the propellant. A knowledge of the exact amount of this volatile matter is of considerable importance in the chemistry of propellants, particularly in relation to the effect on ballistic stability of changes in the volatile matter content arising from variations in the temperatures and humidities under which the propellant is stored and used.

Several attempts have been made to estimate this residual volatile matter in simple nitrocellulose powders containing only cellulose nitrate and diphenylamine, but most of the

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[*] For Part 1, see ANALYST, 1946, **71**, 483–490.

methods described are liable to considerable error. Desmaroux[1] obtained an aqueous solution of the organic solvents by heating under reflux and distilling with aqueous sodium hydroxide solution, and then estimated them by physical methods. The same author[2] later estimated the ethyl alcohol and ether in the aqueous distillate by oxidation with potassium dichromate in acid solution; the ethyl alcohol was separately estimated by the method of Fischer and Schmidt,[3] involving conversion into ethyl nitrite, which was removed by a stream of carbon dioxide and passed into acid potassium iodide solution, the iodine liberated being titrated with thiosulphate. Dalbert[4] also estimated the ethyl alcohol and ether together by dichromate oxidation but replaced the method of Fischer and Schmidt for ethyl alcohol by one depending on surface tension effects. Lalande[5] estimated ether alone by drawing a stream of air through the aqueous distillate, removing the ethyl alcohol in a strongly alkaline solution of potassium permanganate and then absorbing and oxidising the ether to acetic acid with dichromate in diluted sulphuric acid (1+1), the unchanged dichromate being estimated iodimetrically.

A preliminary investigation of these methods indicated that the estimation of mixtures of ethyl alcohol and ether at concentrations of about 0·1 per cent. in aqueous solution was possible with an accuracy to within 1 or 2 per cent. The ethyl alcohol is readily oxidised quantitatively to acetic acid by the method of Szeberenyi[6] by boiling with potassium dichromate in a 1:10 by volume mixture of concentrated sulphuric acid and the aqueous alcohol-ether solution. The ether is not attacked under these conditions and can be estimated by difference after oxidation of another portion of the aqueous solution in a 1:1 by volume mixture of sulphuric acid and solution, which converts both the ethyl alcohol and ether quantitatively to acetic acid; this oxidation is carried out by allowing the mixture to stand at room temperature for 1½ to 2 hours.

In applying this method to the estimation of an aqueous distillate obtained by heating under reflux and distilling a sample of propellant with aqueous sodium hydroxide two major difficulties were encountered. First, a small but appreciable amount of volatile oxidisable matter, from the disintegration of the propellant, distilled with the ethyl alcohol and ether and secondly, in modern nitro-cellulose powders dibutyl phthalate is often present and from it butyl alcohol is formed by the hydrolysing action of the sodium hydroxide; the butyl alcohol distils and interferes in the subsequent dichromate oxidation of the ethyl alcohol and ether.

The first difficulty was met by distilling with sodium hydroxide solution a synthetic mixture of cellulose nitrate, diphenylamine and any other constituents in the amounts present in a 25-g. sample of the propellant and determining the amount of oxidisable matter in the distillate obtained; a correction was then applied for this amount. The presence of butyl alcohol in the distillate, however, required the development of a method of estimating ethyl alcohol, ether and butyl alcohol together in dilute aqueous solution.

THE ESTIMATION OF ETHYL ALCOHOL, BUTYL ALCOHOL AND ETHER IN DILUTE AQUEOUS SOLUTION

Attempts to effect complete hydrolysis of the dibutyl phthalate and subsequent distillation of the whole of the butyl alcohol were unsuccessful; prolonged hydrolysis and distillation did not achieve more than about 80 per cent. recovery of butyl alcohol from known quantities of dibutyl phthalate. Methods of estimating similar simple aliphatic compounds in dilute aqueous solution have been described by Christiansen and Fulmer[7] for mixtures of ethyl alcohol, butyl alcohol and acetone, by Bayly,[8] who investigated the oxidation of aliphatic alcohols in about 0·5 per cent. aqueous solution with potassium dichromate in 45 per cent. sulphuric acid solution; by Skrabal,[9] who claims that with minor modifications the Fischer and Schmidt method (*loc. cit.*)[3] could be applied to an aqueous solution of any simple aliphatic alcohol with an accuracy to within about 1 per cent.; and by Fresenius,[10] who estimated aqueous butyl alcohol solutions by oxidation with dichromate followed by distillation and titration of the acid products in the distillate with sodium hydroxide solution.

Application of Lalande's method—As a first step, the direct estimation of ether in dilute aqueous solutions containing ethyl and butyl alcohols by Lalande's method (*loc. cit.*)[5] was investigated. It was found that an alkaline potassium permanganate solution absorbed both alcohols, whilst the ether, unaffected by passage through this solution, could be absorbed and oxidised quantitatively to acetic acid by potassium dichromate in a 1:1 by volume mixture of sulphuric acid and water. By drawing a slow stream of air through the aqueous solution of the three constituents at 30° to 40° C. for 5 to 6 hours, then through alkaline potassium permanganate solution and finally through the acid potassium dichromate solution, it was established that a quantity of ether of the order of 50 mg. could be estimated with an error of about 1 to 2 per cent.

Oxidation of butyl alcohol—The oxidation of butyl alcohol was next studied and it was found that, under the conditions that convert ethyl alcohol quantitatively into acetic acid in Szeberenyi's method (*loc. cit.*),[6] 1 molecule of butyl alcohol consumes 3 atoms of oxygen; this oxidation procedure is subsequently referred to as the "mild" oxidation method. Attempts to discover other oxidation conditions giving a simple stoichiometric relation between butyl alcohol and oxygen but not affecting ether were unsuccessful, and it was evident that the only immediate possibilities were the complete combustion of the three constituents to carbon dioxide and water, or the quantitative conversion of all three into acetic acid. The former possibility was rejected when the wet combustion method of Williams,[11] employing potassium dichromate or potassium iodate in concentrated sulphuric acid solution, gave erratic results with aqueous solutions of these constituents. Since the oxidation of ether to acetic acid in diluted sulphuric acid (1+1) appeared to be sensitive to any change in the ratio of acid to water, the investigation of the oxidation of butyl alcohol to acetic acid had to be confined to this acid concentration. Employing a dilute aqueous solution of butyl alcohol of known concentration and this concentration of acid, the oxidation was carried out at different temperatures for varying periods of time, and it was finally established that if the temperature was maintained at 0° C. for 24 hours and then raised to and maintained at room temperature for a further 1½ hours, the oxidation proceeded quantitatively to acetic acid; no further change then took place in the potassium dichromate content of the oxidising solution and the amount of potassium dichromate consumed corresponded to 8 atoms of oxygen per molecule of butyl alcohol. Under these conditions of oxidation ethyl alcohol and ether were both quantitatively oxidised to acetic acid. This oxidation procedure was designated the "total" oxidation method. Results of the direct estimation of synthetic aqueous butyl alcohol solutions by the mild and total oxidation procedures are given in Table I. An accuracy of to within 1 or 2 per cent. is evident.

TABLE I

ESTIMATION OF BUTYL ALCOHOL IN AQUEOUS SOLUTION

Butyl alcohol per 100 ml. of aqueous solution taken g.	Type of oxidation	Potassium dichromate consumed g.	Butyl alcohol found g.	Error per cent.
0·0810	Mild	0·319	0·0802	−1·0
0·1084	,,	0·426	0·1074	−1·0
0·1200	,,	0·482	0·1212	+1·0
0·1170	Total	1·224	0·1154	−1·3
0·1170	,,	1·232	0·1162	+0·6
0·1200	,,	1·260	0·1188	−1·0
0·1200	,,	1·258	0·1187	−1·0

Application of methods above—These oxidation methods were applied to synthetic aqueous solutions of ethyl alcohol, butyl alcohol and ether. Three aliquot portions of the solution were separately treated (1) by the "mild" oxidation method, (2) by the "total" oxidation method and (3) by Lalande's method for ether; the quantity of dichromate consumed in each was determined iodimetrically and calculated to 100 ml. of the original solution. The ether was thus obtained directly while the alcohols were obtained indirectly by the following method of calculation.

For 100 ml. of the aqueous solution, let the amounts of potassium dichromate in grams required in the various oxidations be

M. for the preferential oxidation of the two alcohols by the mild oxidation method.
T. for the total oxidation of the three constituents to acetic acid by the total oxidation method.
A. for the oxidation of ethyl alcohol to acetic acid.
B. for the oxidation of butyl alcohol to acetic acid.
C. for the oxidation of ether to acetic acid.

The amounts of dichromate represented by M, T and C are experimentally determined values; the amount of dichromate consumed by the butyl alcohol in 100 ml. of the aqueous solution in the mild oxidation is 3B/8, since one molecule of butyl alcohol requires 3 atoms of oxygen for the mild oxidation and 8 atoms for the total oxidation.

Then for the mild oxidation of 100 ml. of the solution

$$M = A + (3B/8)$$

and for the total oxidation of 100 ml. of the solution

$$T = A + B + C.$$

From these two equations,

$$5A = 3C + 8M - 3T \quad \text{and} \quad 5B = 8(T - M - C)$$

the amount of dichromate equivalent to both alcohols is therefore obtained. The relationship between potassium dichromate and each of the three constituents is given by

1 g. of potassium dichromate ≡ 0·1886 g. of ether
≡ 0·02344 g. of ethyl alcohol
≡ 0·0944 g of butyl alcohol,

from which the amount of each constituent present can be calculated. Results of the estimation of synthetic aqueous solutions of ethyl alcohol, butyl alcohol and ether are given in Table II, and indicate an accuracy to within 1 per cent. for the ether estimation and to within about 2 to 3 per cent. for the alcohols.

TABLE II

ESTIMATION OF MIXTURES OF ETHYL ALCOHOL, BUTYL ALCOHOL AND ETHER IN AQUEOUS SOLUTION

Aqueous solution taken contained: g./100 ml.		Type of oxidation	Potassium dichromate		Found, per 100 ml.		
			Calculated equivalent g.	Amount consumed g.	Ether (direct) g.	Ethyl alcohol (indirect) g.	Butyl alcohol (indirect) g.
Ethyl alcohol	0·0499	Mild	0·624	0·626	0·0504	0·0450	0·1051
Butyl ,,	0·1037						
Ether	0·0500	Total	1·576	1·588			
Ethyl alcohol	0·0502	Mild	0·650	0·646	0·0517	0·0491	0·1100
Butyl ,,	0·1095						
Ether	0·0510	Total	1·644	1·648			
Ethyl alcohol	0·0453	Mild	0·597	0·595	0·0625	0·0435	0·1033
Butyl ,,	0·1015						
Ether	0·0620	Total	1·597	1·610			

In applying this method to estimations on aqueous distillates from propellants containing dibutyl phthalate it was necessary to apply corrections for oxidisable impurities in the distillate, in both the mild and the total oxidation, and these increased the error of the method to the order of about 5 per cent. In the absence of any other suitable method, however, this procedure was regarded as satisfactory for providing a preliminary estimate of the true ethyl alcohol and ether contents of nitrocellulose powders. In the wide variety of powders investigated the ether content was invariably higher than the alcohol content, the former usually ranging from about 0·2 to 2 per cent. and the latter from about 0·1 to 1 per cent.; some old types of powders contained over 5 per cent. of residual solvent.

When the necessity arose for an accurate routine method for the estimation of these volatile constituents certain unsatisfactory features of the method described became apparent. These included the large amount of sample required (25 to 50 g.), which is not always available, and the length of time required for an estimation (2 to 3 days); further, certain special investigations required greater accuracy than was possible with this procedure. As an alternative, the possibility was considered of vaporising the ethyl alcohol and ether in a current of air without decomposition of the propellant and subsequently absorbing and differentially oxidising them in acid dichromate solutions of different concentrations.

THE SEMIMICRO-ESTIMATION OF ETHYL ALCOHOL AND ETHER VAPOURS ENTRAINED IN AIR

Somogyi[12] describes a method for estimating mixtures of ethyl alcohol and ether vapours in air by passing the air first through 9 *N* sulphuric acid, which preferentially absorbs the alcohol, and then through a solution of potassium dichromate in diluted sulphuric acid (1 + 1), in which the ether is absorbed and oxidised to acetic acid; the ethyl alcohol is estimated by subsequently oxidising its solution in sulphuric acid with potassium dichromate. Quantities of the order of 0·25 to 0·025 g. of each constituent were estimated by this method with an accuracy to within about 3 per cent. Komar, Sergunin and Fainberg[13] criticise certain features of this method and apply a slightly modified form of it to the estimation of much smaller quantities of ethyl alcohol and ether in air; their results show that when the amount of ether present is less than 5 mg. the recovery of it is less than 90 per cent., but with larger quantities the method is accurate to within about 3 to 4 per cent.

It was evident that this method might be further modified to include the simultaneous absorption and oxidation of the ethyl alcohol; to this end the conditions necessary for the quantitative oxidation of small amounts of ethyl alcohol (5 to 30 mg.) to acetic acid at room temperatures were investigated. It was found that this could be achieved quite readily with potassium dichromate in diluted sulphuric acid (1+10) in about 1¼ hrs. at ordinary room temperatures. To test the modified method, weighed quantities of ethyl alcohol and ether contained in thin sealed glass tubes were introduced into a 500 ml. flask fitted with an inlet and an outlet tube each carrying a stop cock, the inlet tube reaching to the bottom of the flask. After the stop cocks were closed the tubes were fractured by shaking the flask The flask was placed in an absorption train similar to that shown in the diagram, in the position occupied in the diagram by the three-necked flask. The first absorption spiral tube contained the acid dichromate solution for the oxidation of the ethyl alcohol, the second absorption spiral tube alkaline potassium permanganate solution (prepared as described later) and the third the usual acid dichromate solution for oxidation of the ether. The alkaline potassium permanganate solution was used because in determinations on propellants its presence was effective in absorbing any oxidisable volatile constituents that would not be affected by the weak oxidising solution in the first absorption tube but that might be oxidised by the stronger solution in the third tube. At the head of the absorption train were two conical flasks, the first containing a concentrated chromic acid solution to remove any oxidisable impurities in the air drawn through the train and the second serving as a trap for any acid spray from the first. The last absorption tube in the train was connected to a bottle in which a partial vacuum was maintained by means of a water pump. After all the stop-cocks had been opened a slow stream of air was drawn through the apparatus, carrying the ethyl alcohol and ether vapours into the absorption tubes. After 3 to 4 hours the air flow was stopped, the acid dichromate solutions were washed out and the unchanged potassium dichromate was estimated iodimetrically. Results for several such determinations are given in Table III, and these show that the method is accurate to within about 2 per cent. for quantities of ethyl alcohol and ether not less than about 5 mg.

TABLE III

ESTIMATION OF MIXTURES OF ETHYL ALCOHOL AND ETHER VAPOURS ENTRAINED IN AIR

Taken in mixture					
Ethyl alcohol g.	Ether g.	Ethyl alcohol found g.	Error per cent.	Ether found g.	Error per cent.
0·0365	0·0795	0·0361	−1·1	0·0803	+1·0
0·0343	0·0475	0·0340	−0·9	0·0491	+3·4
0·0227	0·0152	0·0216	−4·8	0·0155	+2·0
0·0187	0·0155	0·0185	−1·1	0·0160	+3·2
0·0097	0·0110	0·0096	−1·0	0·0109	−0·9

APPLICATION TO THE ESTIMATION OF ETHYL ALCOHOL AND ETHER IN PROPELLANTS—

Various methods were considered for removing the ethyl alcohol and ether from nitrocellulose powders free from volatile impurities oxidisable by the acid dichromate solution used in the first absorption spiral tube. It became evident that their complete removal could only be effected by dissolution of the sample of powder in a suitable solvent. Kraus[14] gives a comparison of the solubilities of cellulose nitrates, of nitrogen contents ranging from 10·20 to 12·29 per cent., in 70 different solvents; from this list a selection of the most likely solvents was made and of these, mononitrobenzene proved by far the most suitable.

Dissolution of a 2 to 3-g. sample of a nitrocellulose powder was achieved in about 2 hours at 100° C. Blank determinations on the solvent alone, and also on solutions of the usual constituents of nitrocellulose powders in it, indicated complete absence of interference by volatile oxidisable products. The method adopted therefore was to agitate the sample of powder (2–3 g.) with 25 ml. of nitrobenzene by means of a gas-tight stirrer arrangement in the three-necked flask shown in the diagram. The flask was immersed in a boiling water bath and a stream of air was bubbled through the solution to remove the ethyl alcohol and ether. Other constituents of nitrocellulose powders volatile to some extent at 100° C., *e.g.*, diphenylamine, were almost completely retained by passing the vapours through a condenser before drawing them into the first absorption tube. Acetone and camphor, which are present in some types of powders, were removed by the alkaline potassium permanganate solution (both of these volatile constituents are unaffected by the oxidising solution in the first absorption tube but would be oxidised in the stronger medium in the third absorption tube). To test the method, weighed amounts of ethyl alcohol and ether were dissolved in nitrobenzene and an aliquot portion of the solution was transferred to the three-necked flask; the other constituents of a typical nitro-cellulose powder were added in the amounts present in a 2-g. quantity of sample and the estimation was carried out as indicated above. The time necessary to effect complete removal, absorption and oxidation was about 4 hours. Results given in Table IV indicate errors of 2 to 3 per cent. for both ethyl alcohol and ether. Duplicate results obtained with different types of nitrocellulose powders are given in Table V; excellent reproducibility is evident. The table also includes the result of analysis of one sample containing camphor and one containing acetone in place of ether; the acetone content was 1·10 per cent., but this was completely removed by the alkaline potassium permanganate solution, for no oxidation was found to have occurred in the third absorption tube. The method used for estimating this acetone will be given in Part 3 of this paper. The fact that nitroglycerine does not interfere in the method was demonstrated by carrying out an estimation on a sample of solventless cordite that contained a high percentage of nitroglycerine but no solvent; no oxidation took place in either of the acid dichromate solutions. The method has been found completely satisfactory for all types of propellants. Full details of the solutions and procedure employed are given below.

TABLE IV

ESTIMATION OF MIXTURES OF ETHYL ALCOHOL AND ETHER IN NITROBENZENE SOLUTION

Taken in mixture		Ethyl alcohol found g.	Error per cent.	Ether found g.	Error per cent.
Ethyl alcohol g.	Ether g.				
0·0188	0·0455	0·0186	−1·1	0·0465	2·2
0·0105	0·0142	0·0105	0	0·0144	1·4
0·0105	0·0142	0·0104	−1·0	0·0143	0·7
0·0097	0·0262	0·0097	0	0·0266	1·6
0·0094	0·0164	0·0093	−1·1	0·0170	3·7
0·00323	0·0074	0·0031	−3·7	0·0072	2·7

TABLE V

THE ESTIMATION OF ETHYL ALCOHOL AND ETHER IN PROPELLANT EXPLOSIVES

Sample of propellant	Ethyl alcohol per cent.	Ether per cent.
Modern nitrocellulose powders:		
(1) MA.668	0·08; 0·09	0·57; 0·58
(2) MA.621	0·13; 0·12	0·99; 0·97
(3) Dupont 4825	0·40; 0·40	0·99; 1·01
Neonite (small flake)	0·75; 0·77	0·10; 0·11
German igniter (acetone present)* ..	0·38; 0·39	0·00; 0·00
Celluloid (camphor present)	0·21; 0·20	0·00; 0·00

* Acetone found 1·08 and 1·12 per cent. Estimation to be described later.

DETAILS OF METHOD—

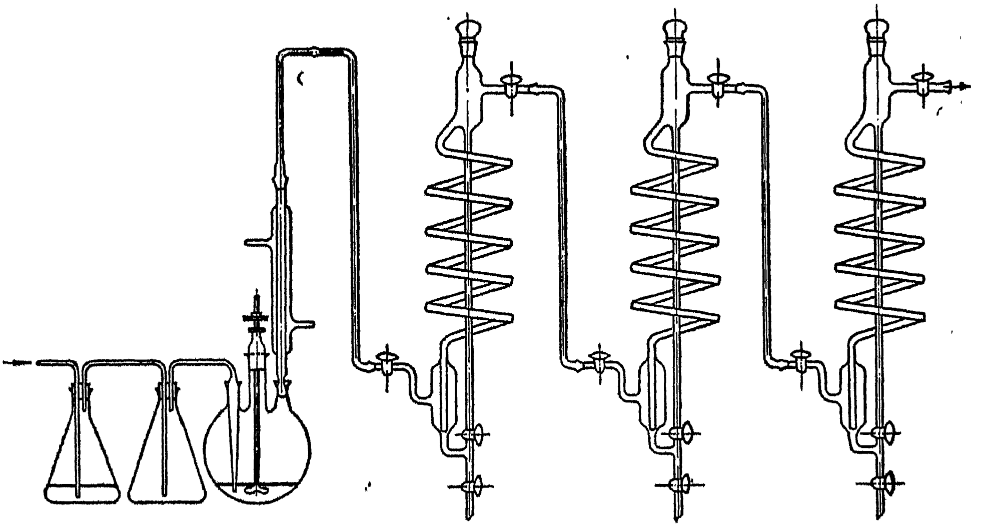
Apparatus—This is shown in the diagram. The absorption spiral tubes have an over-all length of about 18 inches and the diameter of the spiral is about 2 inches; the total internal volume is about 60 ml.

Solutions—To prepare the acid dichromate solution for the oxidation of the ethyl alcohol, transfer 25 ml. of an aqueous solution containing exactly 8 g. of potassium dichromate per litre to a clean dry 100 ml. graduated flask and add 2·5 ml. of concentrated sulphuric acid.

Introduce the bulk of this solution into the first absorption spiral tube shown in the diagram. Retain the flask, as the solution is eventually returned to it.

To prepare the acid dichromate solution for the oxidation of the ether, transfer 25 ml. of an aqueous solution containing exactly 16 g. of potassium dichromate per litre to a clean dry 200 ml. flask and add, while cooling, 25 ml. of concentrated sulphuric acid. Introduce this solution into the third absorption spiral tube shown in the diagram and retain the flask.

To prepare the alkaline potassium permanganate solution boil gently under reflux for 10 hours 104 g. of potassium permanganate and 261 g. of potassium hydroxide with 1 litre of water. After cooling, pour off the supernatant solution and introduce about 30 ml. of it into the second absorption spiral tube.



Procedure.—Crush the propellant sample into small pieces (about 3 mm. across) with a steel pestle and mortar, weigh accurately 2 to 3 g. and add it to 25 ml. of nitrobenzene (AnalaR quality) in the 250 ml. three-necked flask shown in the diagram. (The relative effects of crushing and grinding samples will be described in Part 4 of this paper.) Stir the nitrobenzene solution vigorously and, with the flask immersed in a boiling water bath, draw a stream of air through the solution at the rate of 1 to 2 bubbles per second for 5 hours.

Then stop the flow of air, wash the dichromate solutions back into their respective flasks and make up the volumes to the marks with water. Of the 100 ml. of oxidised ethyl alcohol solution add 25 ml. to a solution of 2 to 3 g. of potassium iodide in about 30 ml. of water and, after 2 minutes, dilute the solution to about 100 ml. with water and titrate the liberated iodine with 0·05 *N* sodium thiosulphate solution, using starch solution as indicator. Treat the oxidised ether solution similarly except that, as the acid concentration is much higher, add the 25-ml. aliquot portion to a solution of the potassium iodide in 100 ml. of water and, after 2 minutes, dilute to about 200 ml. with water and titrate. Calculate the amounts of ethyl alcohol and ether present from the relation to the potassium dichromate given previously (p. 50).

SUMMARY

An account is given of the methods of determining the residual contents of ethyl alcohol and ether in nitrocellulose propellants. A new method is described which employs dissolution of a small sample of the propellant in nitrobenzene at 100° C.; the ethyl alcohol and ether vapours are removed in a current of air and are differentially absorbed and oxidised in acid potassium dichromate solutions of different concentrations. The method is suitable for all normal types of nitrocellulose and nitroglycerine powders and is applicable in presence of the usual constituents of propellants including acetone, camphor, dibutyl phthalate, diethyl diphenylurea and diphenylamine. Arising out of the preliminary investigation of the problem a method is described for the estimation of mixtures of ethyl alcohol, butyl alcohol and ether at concentrations of 0·05 to 0·10 per cent. in aqueous solution.

Acknowledgment is made to Imperial Chemical Industries (Explosives Division) for making available a valuable report in which the earlier attempts at estimating ethyl alcohol and ether in simple nitrocellulose powders were reviewed and critically assessed.

In conclusion, the author wishes to thank Mr. G. L. Hutchinson of the Armaments Research Department for his helpful discussion of the problem and the Director-General of Scientific Research (Defence), Ministry of Supply, for permission to publish this material.

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The Determination of Small Amounts of Aluminium by the Aurintricarboxylate Method

By N. STRAFFORD and P. F. WYATT

In a previous paper[1] we describe a procedure for the separation of very small amounts of aluminium and iron in water, with subsequent determination of the aluminium by means of ammonium aurintricarboxylate. This method gives sufficiently accurate results for waters, but we had occasion to attempt to apply it to the precise determination of small amounts of aluminium in organic compounds, after wet decomposition of 2 g. of the organic material by means of sulphuric, nitric and perchloric acids.[2] The resulting acid solution contained up to 10 mg. of iron and 0 to 300 μg. of aluminium, and two difficulties were met with in the analysis of this solution, *viz.* (1) the amount of iron present was too great, and the conditions were otherwise unsuitable, for complete separation by extraction as ferric thiocyanate, (2) measurements of optical density by means of the Spekker photoelectric absorptiometer showed that the fading of the aluminium aurintricarboxylate colour, rapid at first, then gradually becoming slower, which occurs in slightly basic medium, cannot be controlled sufficiently well to give accurately reproducible results, even when the conditions are carefully standardised.

The first of these difficulties was overcome by converting the iron into its "cupferron" complex, and separating it by extraction with chloroform from a solution containing sulphuric acid at $4N$ to $5N$ concentration. The excess of cupferron reagent can then be extracted sufficiently well to avoid interference with the subsequent aurintricarboxylate reaction. With regard to the second difficulty it was found that after development of the aluminium complex colour as usual in acetate buffered solution, removal of the colour due to excess of reagent could be effected by buffering with ammonium borate to $p\text{H} = 6{\cdot}0 \pm 0{\cdot}5$, and that it was unnecessary to raise the pH to 7·5. With buffering just on the acid side of neutrality in this way, no fading of the aluminium lake occurs, so that results are highly reproducible, and the sensitivity of the method is increased. Under the modified conditions, no interference has been found with up to 10 mg. of calcium, magnesium or phosphate (P_2O_5), or up to 1 mg. of common heavy metals, and it is possible that even larger amounts may be tolerated. Beryllium interferes, giving a colour of intensity similar to that produced by the same weight of aluminium. Iron and copper are removed in the cupferron extraction; it is necessary to ensure thoroughly complete removal of iron, as this element gives a colour about half as intense as that given by aluminium, but small amounts of copper can be tolerated. The procedure recommended for organic compounds is as follows.

REAGENTS—The reagents used are as described in the previous papers.[1,2] The cupferron reagent is a freshly prepared and filtered 6 per cent. solution in water. It must be prepared from material of analytical quality; B.D.H. reagent is suitable. An aqueous solution should be only slightly straw coloured, and a chloroform solution should show no brown discoloration due to decomposition products.

PROCEDURE—Decompose 2 g. of the organic material in a 100-ml. Kjeldahl flask with sulphuric, nitric and perchloric acids,[2] and remove nitric acid by repeated evaporation with water, in the usual manner. Dilute the acid solution until it is 5 *N* to 6 *N* in sulphuric acid, and transfer it to a graduated 50-ml. separating funnel, using the minimum amount of wash water to effect the transference. To this solution, which usually has a volume of 20 to 25 ml., and which should contain not more than 10 mg. of iron, add 2·5 ml. of 6 per cent. cupferron solution, mix well and add 10 ml. of chloroform, B.P. Shake vigorously for 40 seconds, allow to separate, and run off and reject the chloroform layer. Wash with a few ml. of chloroform, without mixing, to displace the drops of strong iron "cupferrate" solution remaining on the surface of the aqueous layer and in the stem of the funnel. Then shake for 30 seconds with 5 ml. of chloroform, allow to separate, and reject the chloroform layer. Add 0·5 ml. of cupferron solution; the turbidity produced should be white and show no colour due to iron. Extract successively with 10 ml. followed by two 5-ml. portions of chloroform, shaking for 30 seconds each time, to remove all but a trace of the free cupferron from the aqueous layer. After each extraction the stopper of the funnel should be rinsed with a little water and the washings added to the contents of the funnel, but the final acidity of the solution should not be allowed to fall below 4 *N*, or traces of aluminium may tend to be extracted.

Transfer the aqueous layer to a 100-ml. conical flask and wash the funnel with small amounts of water. Heat the solution to boiling, and boil for a few seconds to expel any chloroform present; then cool to room temperature. Add one drop of methyl red indicator, and ammonium hydroxide solution, 10 *N*, until the solution is just alkaline. Add dilute hydrochloric acid, 5 *N*, drop by drop, until the solution is just acid, and then 5·0 ml. in excess, followed by 2 drops of saturated bromine water, which should immediately bleach the indicator. Failure of the indicator colour to disappear indicates that the excess of cupferron has not been extracted properly. Finally, add 0·5 ml. of 10 per cent. hydroxylamine hydrochloride solution, to reduce the excess of bromine, and dilute the solution to 100 ml. in a measuring flask (Solution A).

Pipette 20·0 ml. of Solution A into a 100-ml. conical flask and dilute to 30 ml. with distilled water. Add 1·0 ml. of gum arabic solution (5 per cent. in water), 5·0 ml. of ammonium acetate buffer solution, and 2·0 ml. of 0·2 per cent. ammonium aurintricarboxylate solution. Mix well, heat to boiling, and boil gently on a hot plate for 5 minutes. Cool to room temperature, add 4·0 ml. of 0·8 *N* ammonium borate solution, mix, dilute to 50·0 ml. and allow to stand for not less than 5 minutes. Determine the optical density of the test solution and of a reagent blank solution (similarly obtained) on the Spekker photoelectric absorptiometer, using a 4 cm. cell for the range 0 to 10 μg. of aluminium and a 1 cm. cell for the range 10 to 70 μg. of aluminium, with either Chance blue-green glass filters No. 6 (OB2) or Ilford green No. 604 filters. If the amount of aluminium present exceeds 70 μg, take a smaller aliquot portion of Solution A and adjust the acidity so that 1·0 ml. of 5 *N* hydrochloric acid is present, before adding the acetate buffer solution.

Preparation of standard curve—Establish the standard curve required by measuring known amounts of standard aluminium solution (1 ml. ≡ 10 μg. of Al) into 100-ml. conical flasks, adding 1·0 ml. of 5 *N* hydrochloric acid and diluting to 30 ml. Then develop the colour in these solutions as described for the test solution, and determine the optical density of each solution in turn against the first solution (containing no added aluminium) as reference solution. A typical series of results, using a 1 cm. cell, is as follows:

Aluminium present, μg.	10	20	30	40	50	60	70
Optical density:							
1. With Ilford green No. 604 filters ..	0·14	0·285	0·43	0·57	0·72	0·86	1·00
2. With Chance blue-green No. 6 filters	0·10	0·20	0·305	0·405	0·51	0·62	0·71

It is advisable to test Solution A for absence of iron. Take a 50-ml. aliquot portion, add 2 ml. of 1 *M* sodium citrate solution, 1 ml. of 10 per cent. thioglycollic acid solution, and finally 5 ml. of 10 *N* ammonium hydroxide, and mix. The solution should show no trace of pink colour due to iron.

Application to water samples—For waters, we recommend that the procedure previously described[1] be modified as follows. After extraction of the iron as thiocyanate, boil the solution to remove ether, cool and dilute to 50 ml. in a measuring flask. Take a 20-ml. aliquot portion and determine its acidity by titrating with *N* sodium hydroxide to methyl red indicator. Reject this test, take a second 20-ml. aliquot portion and add the same amount of *N* sodium hydroxide as was required by the first test. Treat the reagent blank similarly. Add to blank and test 1·0 ml. of 5 *N* hydrochloric acid and then develop the colour of the aluminium complex exactly as described above. The optical density of the solution may be measured by the Spekker absorptiometer, or the colour may be matched with the iron thioglycollate colour disc in the B.D.H. Lovibond Nessleriser. With the above modified procedure, the disc reading (μg. of Fe) divided by 20 represents μg. of aluminium, and if a 20/50 ml. aliquot portion is taken, 8·0 μg. of iron (disc reading) ≡ 1 μg. of aluminium in the total solution which corresponds to 0·10 parts of aluminium per million on a 10-ml. sample or 0·04 parts per million on a 25-ml. sample, as before.[1]

REFERENCES

1. Strafford, N., and Wyatt, P. F., ANALYST, 1943, **68**, 319.
2. Strafford, N., Wyatt, P. F., and Kershaw, F. G., *Ibid.*, 1945, **70**, 232.

IMPERIAL CHEMICAL INDUSTRIES LIMITED
ANALYTICAL LABORATORIES
BLACKLEY, MANCHESTER, 9

October, 1946

A Colour Reaction of Certain Classes of Azo Dyes with Copper

BY W. A. ALEXANDER

A CHANCE observation of the behaviour of the dyestuff Bordeaux B with dilute copper solutions led to an investigation from which it appears that a certain class of dyes can be distinguished by means of the characteristic reaction described below. The reaction also serves, under certain conditions, as a sensitive test for copper.

TEST—Add a few drops of a dilute (about 0·05 per cent.) aqueous solution of the dye to be tested to 10 ml. of a weakly acid solution of a copper salt (0·0001 *M* to 0·001 *M*), and then add sufficient sodium acetate to bring the *p*H to about 8·5. Carry out a control test—without copper. If the dye belongs to the reacting class, a distinct colour change will be noticed. Most of the reacting dyes are pink or red—and with these the colour becomes yellow-brown; in two instances the colour change in presence of copper is from blue-violet to blue-grey. Some of the reacting dyes, *e.g.*, Bordeaux B, are more sensitive than others; these sensitive dyes may be used as reagents for copper. When so used, the reaction provides a delicate test for that metal, and is capable of detecting as little as 1 μg. of copper in 10 ml., *i.e.*, a concentration of 1 in 10,000,000. In testing for copper, the solution must be dilute; if sufficiently concentrated for cupric hydroxide to be precipitated the colour change is not observed. In testing for copper, too, the amount of dye solution taken should be the minimum necessary to give a distinct colour to the control; if too much be taken, the excess tends to mask the colour change.

The test is particularly suitable for detecting copper in water for domestic use, where there is little interfering material.

INTERFERENCE—The remarks under this heading, of course, apply either when the copper ion is used to distinguish certain dyes or when a sensitive dye is used for a qualitative test for copper.

High concentrations of all salts (over 1·0 *M*) lower the sensitivity of the reaction somewhat. This applies to substances such as sodium chloride and potassium sulphate. Borates, fluorides, chromates, nitrites and nitrates, at concentrations below 0·1 *M*, do not interfere. Phosphates and silicates mask the test somewhat, but not at concentrations below 0·001 *M*. Cyanide, even in traces, masks the test completely.

Of the metals tested, only nickel reacts similarly to copper—but only when the *p*H is greater than 8·5 and the concentration of the nickel greater than 1 in 200,000. Manganese, cobalt, lead, silver, barium, strontium, calcium, magnesium, cadmium and arsenic do not interfere at low concentrations. Antimony, tin and bismuth do not interfere when the reagent is added after the solution has been filtered at *p*H 8·5 (test for copper). Chromium, aluminium and ferric iron adsorb the dye when their hydroxides are precipitated in its presence—there is no colour change, however, and traces do not interfere. Ammonium ion lowers the sensitivity and causes the formation of a dark brown in place of a yellow-brown tint with sensitive dyes of a red colour.

REACTIVE DYES—Of the dyes examined—representative of several classes—only certain azo dyes are reactive. and the conclusions drawn may be summarised as follows.

(1) Copper-sensitive dyes contain an azo group linked in the 2 or 1 position to sulphonated α- or β-naphthol respectively.

(2) Reactive dyes derived from α-naphthol are substituted in the 4 and/or 5 positions with SO_3' groups, while those derived from β-naphthol are substituted thus in the 3 and 6 positions.

(3) In both classes, the 8 position may be occupied by SO_3' or OH.

(4) Dyes that are insensitive include those closely related azo dyes derived from α- or β-naphthol, in which the above order of substitution is reversed or substituents are not present.

(5) The remaining part of the dye structure appears to have little influence on the reaction with copper.

Tables I to IV illustrate these conclusions, together with the structure of the dyes. Of the insensitive dyes, only those closely related to the sensitive class are shown. Table I shows the reactive dyes derived from α-naphthol and Table II the inactive dyes from the same source. Table III gives the reactive dyes derived from β-naphthol—these are rather more sensitive as a class than those in Table I and include the very sensitive Bordeaux B, No. 88. Table IV shows the non-reacting β-naphthol dyes. The numbers in all four tables refer to the Colour Index. Only one exception to the substitution order mentioned above was noted, No. 54 (Table I), which has sulphonic groups in the 3 and 6 positions, whereas one would expect it to be a 4:5 or 4:8 disulphonate for a reacting α-naphthol dye. This may, however, be due to the presence of an excessive proportion of these reactive isomers.

With all the reactive dyes except two the colour change is similar—from red to yellow-brown; the two exceptions are Azo Acid Violet AL (Table I) and Azo Acid Blue (Table I, No. 59), with both of which the change is from violet to blue-grey.

MECHANISM OF REACTION—Several workers have studied the lakes formed by the interaction of certain metals with azo and other dyes. Many of the former have been shown by Drew and Landquist,[1] Beech and Drew,[2] and Boyle, Cumming and Steven,[3] to be inner co-ordination complexes. When copper is the metal, these are frequently soluble in water and amorphous in structure. Such compounds are usually formed under alkaline conditions; for example, the authors named have shown that if sulphonic groups be present a sulphonate may be first formed, and no inner co-ordination results until the sulphonic groups are neutralised. Again, a great many of the compounds which have been recorded are brown, or reddish-brown in the solid phase. Lake formation, too, generally requires the presence of a hydroxyl group in the *ortho* position with respect to the azo group.

These facts indicate that the delicate colour change noted at extreme dilution may be due to the formation of a copper lake.

There are, however, one or two facts that are not so readily reconciled with this view.

Lake formation, it appears, can also occur with compounds having an *ortho* carboxyl or amino substituent in place of the hydroxyl group, and such compounds do not react with copper at extreme dilution in the manner described. Again, the authors cited make no deductions with respect to substitution rules and lake formation similar to those given above; copper lakes are obtained from a number of *ortho* hydroxy azo dyes whether derivatives of naphthalene or not, but only certain sulphonated naphthol dyes give the characteristic reaction with copper. For example, Boyle, Cumming and Steven show that dye No. 151 (Table IV) forms a reddish-brown copper lake, but it does not react characteristically at extreme dilution.

A comparison, at great dilution and equivalent concentrations, between the colours obtained on dissolving the solid Bordeaux B - copper lake and that resulting from addition of the dye to the copper ion at great dilution showed that the former was considerably darker (browner) in shade.

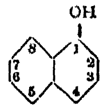


TABLE I: REACTIVE DYES DERIVED FROM α-NAPHTHOL

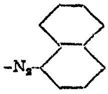
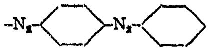
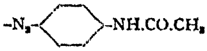
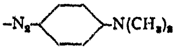
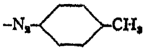
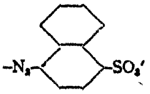
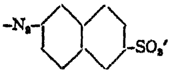
Name of dye or Colour Index No.	Positions of substituents							
	1	2	3	4	5	6	7	
Fast Red VR	-OH		—	—	$-SO_3'$	—	—	—
Azo Acid Violet AL	-OH		—	$-SO_3'$	—	—	—	-OH
No. 54	-OH		$-SO_3'$	—	—	$-SO_3'$	—	—
No. 59	-OH		—	$-SO_3'$	—	—	—	-OH
No. 74	-OH		—	$-SO_3'$	—	—	—	—
No. 179	-OH		—	$-SO_3'$	—	—	—	—
No. 194	-OH		—	$-SO_3'$	—	—	—	—

TABLE II: NON-REACTIVE DYES DERIVED FROM α-NAPHTHOL

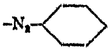
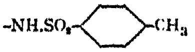
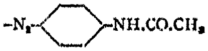
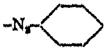
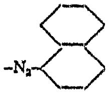
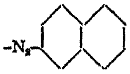
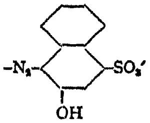
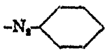
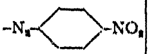
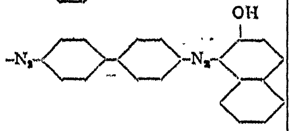
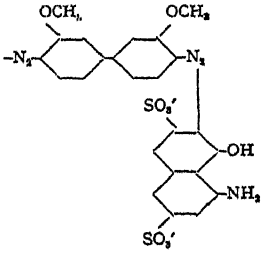
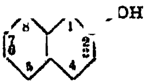
No. 32	-OH		$-SO_3'$	—	—	$-SO_3'$	—	
No. 57	-OH		$-SO_3'$	—	—	$-SO_3'$	—	-NH.CO.CH_3
No. 77	-OH		$-SO_3'$	—	—	$-SO_3'$	—	—
No. 85	-OH		$-SO_3'$	—	—	$-SO_3'$	—	—
No. 91	-OH		$-SO_3'$	—	—	$-SO_3'$	—	—
No. 201	-OH		—	—	—	—	—	—
No. 246	-OH		$-SO_3'$	—	—	$-SO_3'$		$-NH_2$
No. 387	-OH		$-SO_3'$	—	—	$-SO_3'$	—	$-SO_3'$
No. 520	-OH	 OCH_3 OCH_3 $-N_2-$ $-N_2$ SO_3' -OH $-NH_2$ SO_3'	$-SO_3'$	—	—	$-SO_3'$	—	$-NH_2$

TABLE III: REACTIVE DYES DERIVED FROM β-NAPHTHOL



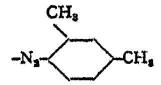
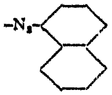
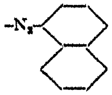
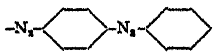
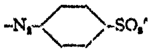
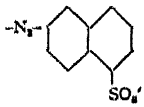
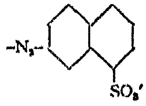
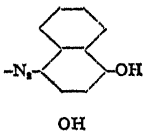
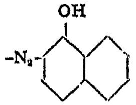
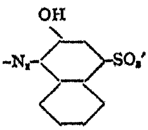
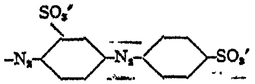
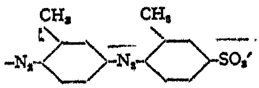
Colour Index No. of dye	Positions of substituents 1	2	3	4	5	6	7	8
No. 79		-OH	$-SO_3'$	—	—	$-SO_3'$	—	—
No. 88		-OH	$-SO_3'$	—	—	$-SO_3'$	—	—
No. 89		-OH	—	—	—	$-SO_3'$	—	$-SO_3'$
No. 252		-OH	—	—	—	$-SO_3'$	—	$-SO_3'$

TABLE IV: NON-REACTIVE DYES DERIVED FROM β-NAPHTHOL

Colour Index No. of dye	1	2	3	4	5	6	7	8
No. 151		-OH	—	—	—	—	—	—
No. 196		-OH	—	—	—	—	—	—
No. 196		-OH	—	—	—	—	—	—
No. 201		-OH	—	$-SO_3'$	—	—	—	—
No. 201		-OH	—	$-SO_3'$	—	—	—	—
No. 202		-OH	—	—	—	—	—	—
No. 280		-OH	—	—	—	—	—	—
No. 286		-OH	—	—	—	—	—	$-SO_3'$

The formation of the co-ordination compounds studied by the authors cited requires heating in concentrated solutions, and it is difficult to see how the rapid reaction at extreme dilution described above can be due entirely to lake formation of this type. It seems more reasonable to suppose that certain dyes are adsorbed by colloidal cupric hydroxide.

REFERENCES

1. Drew, H. D. K., and Landquist, J. K., *J. Chem. Soc.*, 1938, 292.
2. Beech, W. F., and Drew, H. D. K., *Ibid.*, 1940, 608.
3. Boyle, J. L., Cumming, W. M., and Steven, A. B., *J. Roy. Tech. Coll., Glasgow*, 1940, 617.

THE ROYAL TECHNICAL COLLEGE
GLASGOW

July, 1946

Notes

THE DETERMINATION OF COPPER IN FOOD

IN a study of methods for the determination of copper in food, it was found that certain difficulties associated with the preparation of samples by dry-ashing could be overcome and that the procedure for photometric absorption measurement, when using sodium diethyl dithiocarbamate as the colouring agent, could be simplified.

ASHING—Whilst a dry-ashing procedure is more convenient and less liable to contamination from reagents than a wet-ashing one, it is generally accepted[1,2,3] that it gives low recoveries of copper if the ashing temperature exceeds 500° C. This comparatively low temperature not only needs control but may cause a difficulty in obtaining reasonably complete ashing of some products.

The generally accepted explanation of the loss of copper is that it is due to formation of an acid-insoluble compound and not to volatilisation.[1] Piper[4] has suggested that the compound is a complex silicate of copper, and support for this is given by Bailey and McHargue,[5] who obtained lower results when using silica dishes for dry ashing than when they ashed by the wet method; by Comrie,[6] who obtained greater losses when using old silica dishes than when new ones were used, and by Van Niekerk,[7] who observed slagging of copper salts during dry ashing when they came into contact with the sides of the silica dishes he used.

It is the general practice to extract the ash with hydrochloric acid and to determine the amount of copper present in the extract, and an alternative explanation of the loss of copper is that it is due to the reduction of the copper compounds present to metallic copper by the carbon which is necessarily formed during dry ashing. This reduction occurs at relatively low temperatures[8] and, as copper is only feebly attacked by hydrochloric acid,[8] losses are to be expected when this acid alone is used for extraction. If this view is correct it is to be expected that the use of a more suitable extracting acid will result in a full recovery of copper.

The results of some recovery experiments with a farinaceous product ashed at 650° to 800° C. are given in Table I. These clearly show that the presence of nitric acid in the extracting acid results in a much more complete recovery of added copper.

TABLE I

RECOVERY OF COPPER FROM ASH WITH DIFFERENT EXTRACTING ACIDS

Hydrochloric acid			Mixture of hydrochloric and nitric acid		
Copper added µg.	Copper found µg.	Recovery %	Copper added µg.	found µg.	Recovery %
—	8·0	—	—	21·6	—
50	13·2	10·4	50	70·2	97·2
50	12·6	9·2	50	70·6	98·0
50	9·2	2·4	50	67·0	90·8
50	9·2	2·4	50	71·2	99·2
50	11·2	6·4	50	67·0	90·8
50	11·2	6·4	50	68·8	94·4

It is possible to obtain consistently satisfactory recoveries of added copper when hydrochloric acid alone is used to extract the ash, provided the ashing temperature is kept at 450° to 490° C. This does not, however, show that the copper already present in the product is completely recovered, nor are the satisfactory recoveries obtained when nitric acid is used a proof of the accuracy of the nitric acid method. A series of analyses by the two dry-ashing methods were, accordingly, run in parallel with a standard wet-ashing method.[1] The results, given in Table II, whilst showing excellent agreement between the nitric acid and the wet ashing methods, show also that the hydrochloric acid method is not reliable.

TABLE II

DETERMINATION OF COPPER IN DIFFERENT PRODUCTS

Product	Dry-ashing		Wet-ashing
	HCl extraction	$HCl + HNO_3$ extraction	
	p.p.m.	p.p.m.	p.p.m.
Peas	6·3	7·4	7·4
	6·3	7·3	7·4
	6·4	7·3	7·3
	6·3	7·4	7·4
Treacle pudding ..	2·1	6·0	5·9
	2·0	6·0	6·0
	2·1	6·0	6·1
		6·1	5·9
Mixed pickles ..	5·4	8·9	8·8
	5·2	9·1	9·0
	5·7	9·1	8·8
	5·6	9·0	9·0

When nitric acid has been used in the treatment of ash,[9,10,11] it has either been used as a part of what is virtually a wet-ashing procedure carried out in open dishes rather than in a flask[9] or because a subsequent electrolytic separation of copper makes the presence of hydrochloric acid undesirable.[10, 11] In none of these instances is the nitric acid used to ensure a more complete extraction of the ash than would be obtained if hydrochloric acid alone is used.

Measurement of photometric absorption—The use of nitric acid often results in a yellow-coloured extract. The colour varies from sample to sample and it is, therefore, necessary to measure or allow for the blank of each extract. With food products containing low amounts of copper, a solution of the extract as concentrated in respect of copper as is practicable should be used and the dilution necessary if separate aliquots of the extract are taken for blank and colour measurement should be avoided if possible.

TABLE III

PHOTOMETRIC ABSORPTION OF A COPPER SOLUTION* AFTER ADDITION OF VARIOUS AMOUNTS OF SODIUM DIETHYLDITHIOCARBAMATE

Weight of sodium diethyldithiocarbamate added, mg.	Photometer reading†
2	50·8
5	57·0
10	69·0
20	82·8
50	82·8
75	83·2
100	82·6
250	83·0
500	85·4‡

* Containing 94 µg. of copper in 50 ml.
† Water setting 47·8.
‡ Slight cloudiness in the solution.

As can be seen from Table III, the absorption of a copper solution is independent of the amount of sodium diethyldithiocarbamate present once the maximum absorption has been reached. It is, therefore, practicable, by using the solid reagent for colour development, to measure the blank and the copper absorptions on the undiluted extract.

There is apt to be waste when the pure solid reagent is used, as, at most, only 20–30 mg. are required for full colour development. Further, if too much is used, the test solution tends to be cloudy owing to incomplete solution of the diethyldithiocarbamate. It is therefore suggested that the reagent be diluted with a salt which is readily soluble and is without influence on the copper colour. A 1:25 mixture with sodium chloride will be found to be a convenient one to use.

METHOD—Dry-ash a suitable sample of the product in a silica basin in the normal manner, igniting at 600° to 850° C. Extract the ash by warming it with 5 to 10 ml. of a mixture of 2 volumes of diluted hydrochloric acid (1+1) to 1 volume of diluted nitric acid (1+1), both of analytical reagent purity.

Transfer the extract to a 100-ml. beaker, add about 2 g. of A.R. citric acid or ammonium citrate and 2 ml. of 1 per cent. gum arabic solution, neutralise with A.R. ammonium hydroxide (sp.gr. 0·880), and add 1 to 2 ml. in excess. Allow the solution to cool and transfer, with filtration if necessary, to a 50- or 100-ml. volumetric flask and make it up to the mark with water.

Set the photometer to a predetermined setting with a part of the contents of the flask, using Ilford 601 spectrum violet filters. Transfer another part to a beaker or, using the solution already in the photometer cell, add about 0·5 g. of a 1:25 mixture of sodium diethyldithiocarbamate and A.R. sodium chloride. Stir to dissolve the mixture and measure the absorption of the solution. The increase is that due to the copper colour.

Calibrate the photometer by means of a standard solution prepared from A.R. cupric sulphate or pure copper foil, with the same size of measuring cell as that used in the test, and measuring the absorption as directed above.

Thanks are due to the Metal Box Co., Ltd., for permission to publish, and to Dr. H. Liebmann for invaluable advice and constructive criticism.

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RESEARCH DEPARTMENT
THE METAL BOX CO., LTD.
ACTON, LONDON, W.3

JOHN H. HIGH
October, 1946

NOTE ON THE ASSAY OF SOLUTIONS OF *d*-TUBOCURARINE CHLORIDE FOR INJECTION

THE renewed interest in *d*-tubocurarine chloride, resulting from its extensive use for muscular relaxation during surgical operations,[1] has made it desirable that analysts should be familiar with chemical and physical methods which, apart from the fundamental biological assay, may be used for the standardisation of injections. In our laboratories it has been found that the colorimetric and polarimetric methods, described below, may be used for the routine examination of solutions, which usually contain 1 per cent. w/v of crystalline *d*-tubocurarine chloride.

POLARIMETRIC ASSAY—Crystalline *d*-tubocurarine chloride, $C_{38}H_{44}O_6N_2Cl_2\,5H_2O$, possesses a specific rotation of +190° (*C* 1·0 in water) equivalent to $[\alpha]_D^{20} + 215°$ for the anhydrous salt. The specific rotation in water, however, is practically independent of the concentration and consequently the optical rotation of a solution is a linear function of its *d*-tubocurarine content. This property forms a useful method for the assay of solutions, the optical rotation of an aqueous 1 per cent. w/v solution of crystalline *d*-tubocurarine chloride being +1·90°.

COLORIMETRIC ASSAY—*d*-Tubocurarine chloride yields with the Folin-Ciocalteu phenol reagent[2] a brilliant blue colour, which will detect as little as 0·01 mg. of the alkaloid. The reaction may be made quantitative when carried out under the following conditions.

Standard solution—Dissolve 10 mg. of crystalline *d*-tubocurarine chloride, $C_{38}H_{44}O_6N_2Cl_2.5H_2O$, in water and make up to 100 ml.

Test solution—Dilute the solution under examination to approximately the same concentration as the standard solution.

Reagent—Folin-Ciocalteu phenol reagent.[2] For use, dilute 1 volume of strong stock solution with 2 volumes of water.

Colour reaction—Measure 2 ml. of the standard or test solution into a 25-ml. glass-stoppered measuring cylinder, add 3 ml. of reagent and adjust the volume to 25 ml. with water. Add 2 ml. of 20 per cent. w/v sodium carbonate solution, mix, and heat a suitable volume (5 ml.) in a test tube placed in a boiling water bath for 3 minutes Cool the reaction mixture.

Prepare under identical conditions reaction mixtures from the standard and test solutions and compare them in a suitable colorimeter. From the readings obtained calculate the *d*-tubocurarine chloride content of the test solution.

A phenol, which may be present as an antiseptic in a solution for injection, will interfere with the colorimetric assay, and either a correction must be applied to the colorimeter reading or the phenol must be removed before addition of the reagent. When dealing with preparations of uncertain composition the analyst must proceed on the assumption that a phenolic antiseptic is present. For example, if a single 1-ml. ampoule, containing 10 mg. of *d*-tubocurarine chloride, is available, the following procedure should be adopted. The ampoule is opened, a portion of the solution (about 0·5 ml. required) is transferred to a 50-mm. micro-polarimeter tube and the optical rotation is determined. This polarimetric assay consumes no material and the solution is returned to the ampoule, from which 0·5 ml., accurately measured with a pipette or a micrometer syringe, is transferred to a separating funnel containing 25 ml. of water, and the resulting diluted solution, rendered faintly acid if necessary, is extracted with three portions (2·5 ml.) of chloroform. The chloroform extracts are discarded and the aqueous layer run into a 50-ml. standard volumetric flask to which the aqueous washings from the separating funnel are added to adjust the volume to 50 ml. The solution is well shaken and used for the colorimetric assay. After the polarimetric and colorimetric work has been completed sufficient of the original solution will remain in the ampoule for a biological examination, if this is required.

The methods described in this communication have yielded results correct to within ± 5 per cent. It might be feared that the polarimetric assay would be unreliable on account of possible racemisation of the *d*-tubocurarine but, in our experience, solutions of the chloride undergo little change when subjected to the usual process of heat sterilisation.

I am indebted to the Directors of The Wellcome Foundation for permission to publish this information.

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THE CONTROL LABORATORIES
WELLCOME CHEMICAL WORKS
DARTFORD, KENT

G. E. FOSTER
December, 1946

OXALIC ACID EXTRACTANT IN VITAMIN C ASSAY

SOLUTIONS of oxalic acid have advantages over metaphosphoric or trichloroacetic acid as extractants for vitamin C in being cheaper and more stable, and avoiding precipitation of protein, whilst effectively inhibiting the action of ascorbic acid oxidase. In a collaborative investigation on vitamin C in tomatoes, involving the preparation of large volumes of extracts, the opportunity was taken to compare the results obtained on a given fruit with oxalic and metaphosphoric acids respectively, using sufficiently large numbers of fruit to obtain averages in which the sampling error was reduced to a low level. With 1 per cent. and 2 per cent. solutions of oxalic acid the average results were respectively 98 and 99 per cent. of those obtained when 5 per cent. metaphosphoric acid solution was used. The *p*H of 10 to 40 per cent. extracts in 1 per cent. oxalic acid solution was 1·8 to 2·0 and that in 2 per cent. solutions 1·1 to 1·2. The *p*H of the tomato flesh was 4·5 to 5·5 and that of the skin 7 to 8. Control experiments showed the presence of much ascorbic acid oxidase. The extracts in metaphosphoric acid showed no appreciable loss after a week's storage in the refrigerator in brown bottles filled with nitrogen—the routine procedure applied to metaphosphoric acid extracts. With these precautions, oxalic acid can safely be used as an extractant in vitamin C assays on tomatoes and similar large fruit. It is, however, less suitable for extracting unripe walnuts containing much "apparent vitamin C" of which the dye titration value depends on the *p*H (*cf. Nature*, 1946, **158**, 133).

OVALTINE RESEARCH LABORATORIES
KING'S LANGLEY, HERTS

FRANK WOKES
November, 1946

Official Appointments

PUBLIC ANALYST APPOINTMENTS

Notification of the following appointments of Public Analysts has been received from the Ministry of Health since the last record in The Analyst (1946, 71, 586).

Public Analysts	*Appointments*
Branson, Victor Cecil (Deputy)	County Borough of Hastings.
Cox, Henry Edward	Isles of Scilly.
Hawkins, Ernest Stephen	Boroughs of Folkestone, Gravesend, Maidstone and Margate.
Jenkins, Daniel Ceiriog Evans	Borough of Colne.
Lovett, Thomas Whittaker (Deputy)	County Borough of Rochdale.
Moir, Daniel Donald	Urban District of Coulsdon and Purley.

For the following particulars of Public Analyst Appointments in Scotland, held at January 1st, 1947, we are indebted to the Department of Health for Scotland.

Public Analyst	*Appointments*
Dargie, Andrew	*Counties:* Angus, Fife, Perth and Kinross. *Burghs:* Arbroath, Dundee, Dunfermline and Kirkcaldy.
Hawley, John William, and **Wilson**, William (additional)	*Counties:* Dumfries, Kirkcudbright and Wigtown. *Burgh:* Dumfries.
Jamieson, Archibald R., and **Herd**, Magnus (additional)	*Burgh:* Glasgow.
McKean, John Brown	*Counties:* Argyll and Bute. *Burghs:* Greenock, Port Glasgow, Paisley, Dumbarton and Motherwell and Wishaw.
Mundy, Mrs. Lilian M.	*Counties:* Ayr and Renfrew. *Burghs:* Ayr and Kilmarnock.
Robb, Marshall J., and **Ritchie**, John E.	*Counties:* Aberdeen, Banff, Caithness, Kincardine, Ross & Cromarty and Sutherland.
Scott-Dodd, Alexander	*Counties:* Berwick, Inverness, Moray and Nairn, Orkney, Roxburgh, Selkirk and Zetland. *Burghs:* Edinburgh and Inverness. *Small Burghs:* Dunbar, North Berwick and Peebles.
Thin, Russell Gibson	*Counties:* Clackmannan, East Lothian (excluding Burghs of Dunbar and North Berwick), Midlothian, Peebles (excluding the Burgh of Peebles) and West Lothian.
Thomson, Robert Tatlock, and **McKean**, John Brown	*Counties:* Dunbarton and Stirling. *Burghs:* Airdrie, Clydebank, Coatbridge, Falkirk, Hamilton, Perth, Rutherglen and Stirling.
Weir, Alexander B., and **Macdonald**, Joseph (assistant)	*Burgh:* Aberdeen.
Wilson, Andrew, and **McKean**, John Brown	*County:* Lanark.

Ministry of Food

STATUTORY RULES AND ORDERS*

1946—No. 2046. The Meat Products, Canned Soup and Canned Meat (Control and Maximum Prices) (Amendment No. 3) Order, 1946. Dated December 5, 1946. Pp. 6. Price 2d.

This Order amends further the Meat Products, Canned Soup, and Canned Meat (Control and Maximum Price) Order, 1946, *as previously amended by S.R. & O.,* 1946, *Nos.* 1355, 1542, *and* 1727. *The principal amendments are concerned with maximum prices. Soups, both meat and vegetable, have been entirely removed from the scope of the Order.*

This Order came into force on the 9th of December, 1946.

— **No. 2124. The Feeding Stuffs (Regulation of Manufacture) Amendment No. 10) Order, 1946. Dated December 13, 1946.** Pp. 6. Price 2d.

The purpose of this amending Order is to prescribe a formula for the manufacture of a new cattle food to be known as National Cattle Food No. 2; to prescribe a minimum wheat by-products content and to decrease the minimum cereals content of all National Compounds except National Baby Chick Food. The Order also increases the minimum percentage of fish meal to be included in

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Italics signify changed wording.

certain pig and poultry compounds and makes a minor alteration in the grade of veterinary cod liver oil to be included in those compounds of which cod liver oil is a required ingredient.

The analytical requirements of the various compounded rations are specified.

This Order came into force on the 21st of December, 1946.

1946—No. 2169. The Labelling of Food Order, 1946. Dated December 19, 1946. Pp. 20. Price 5d.

This Order revokes and substantially re-enacts in a consolidated form the Labelling of Food (No. 2) Order, 1944, and its amending Orders. New provisions have been introduced, particularly as to the labelling of intoxicating liquors. Cocktails, cordials, processed peas and vitamins are now included.

The general purport of the Order is:—

- (*a*) *to provide that all intoxicating liquors sold in bottle be labelled with an indication of their true nature, their country of origin and, with certain exceptions, their strength (Article 2 (5));*
- (*b*) *to provide that intoxicating liquors for which tonic, restorative or medicinal properties are claimed, be labelled with a statement indicating the quantity of the ingredients on which the claim is based (Article 2 (5));*
- (*c*) *to provide that liquids packed in advance by a retailer on the premises where they are sold be labelled with an indication of their true nature (Article 2 (7));*
- (*d*) *to include provisions as to liquors sold in bottle which may be labelled under descriptions which suggest that they are or resemble spirits, cocktails, sweetened liqueurs or alcoholic cordials (Article 4);*
- (*e*) *to make special provision as to the labelling of canned processed peas (Article 6);*
- (*f*) *to provide that a statement of ingredients be included on the labels of pickles, canned soups and canned meats which are now no longer exempted from this requirement by inclusion in the First Schedule to the Order.*

The Order contains four Schedules, as under:

1. Foods exempt or partly exempt from Article 2 of the Order.
2. Part I Vitamins. Part II Minerals. Units to be used on labels.
3. Dates of revocation of provisions of the Labelling of Food (No. 2) Order, 1944, as *now* amended.
4. Dates of coming into force *of the various articles and paragraphs, between the* 1*st of February,* 1947, *and the* 1*st of January,* 1948.

Amongst new regulations of general interest, the Order requires that:

1. *All undistilled excisable, fermented liquor, not made from freshly gathered grapes, shall bear a prescribed label stating the description of fruit, fruits or fruit products used by the manufacturer in the process of fermentation and also the minimum alcoholic content, expressed either as a percentage of alcohol by volume or as a percentage of proof spirit. In addition, liquor "not made from fruit" is to be labelled with these quoted words, and brandy, gin, rum and whisky that has been diluted with water to an alcoholic strength of less than 65 per cent. of proof spirit is required to bear a label to that effect and also a declaration of its minimum alcoholic strength.*
2. *Brandy, the alcoholic strength of which has fallen below 65 per cent. of proof spirit solely on account of maturing in the cask is not required to be labelled "diluted," but must bear a statement of its minimum alcoholic strength, expressed either as a percentage of proof spirit or in degrees proof.*
3. *On all labels,* the declaration shall be printed in dark block type not less than ⅛ inch in height upon a light coloured ground and shall be enclosed by a surrounding line and no matter other than that hereinbefore described shall be printed within such surrounding line, provided that in the case of brandy, gin, rum and whisky, the alcohol content of which is not less than 65 per cent. proof spirit and of any other intoxicating liquor the alcohol content of which is not less than 40 per cent. proof spirit, it shall be sufficient for the declaration to be printed in dark black type not less than ⅛ inch in height upon a light coloured ground or in light block type not less than ⅛ inch in height upon a dark coloured ground in the following form:

   (Z) PROOF

   inserting at (Z) the figure which represents the percentage of proof spirit.
4. *A cocktail is required to contain not less than* 40 *per cent. of proof spirit,* but so long as no name or description usually associated with a cocktail containing spirits or with a sweetened liqueur is used, nothing shall prevent the use of:
   - (i) the description "Wine Cocktail" for a product which is derived from wine obtained by the fermentation in the district of its origin of the juice of freshly gathered grapes and which contains not less than 35 per cent. proof spirit, or
   - (ii) the description "British Wine Cocktail" or the words "wine cocktail" immediately preceded by the name of the fruit or other saccharine material from which the product is derived, for a product which is made from wine other than that referred to in the preceding sub-paragraph and which contains not less than 24 per cent. proof spirit, or
   - (iii) the description "Alcoholic Cordial" for a suitably flavoured compounded spirit which has been rendered sweet and viscous only by addition of sucrose, dextrose or invert sugar and not by the use of any other ingredient and which contains not less than 10 per cent. proof spirit, or
   - (iv) the description "Non-Alcoholic Fruit (or Vegetable) Juice Cocktail" for a non-alcoholic product consisting of undiluted fruit or vegetable juice and ready for consumption without dilution.

No person shall sell or have in his possession for sale any canned peas which have been dried, soaked or otherwise processed prior to canning which are described in a label attached to or printed on the wrapper or container

(*a*) as "peas," unless the word "peas" wherever it appears on the label is immediately preceded by the word "processed" printed in such a manner as to be substantially as conspicuous as the word "peas."

(*b*) as being "fresh," or "garden" or "green," or by the use of any word which may indicate either directly or by ambiguity, omission or inference, that the peas are other than peas which have been dried, soaked or otherwise processed prior to canning.

This Order details the special requirements for foods in which the presence of vitamins or minerals is claimed on labels or in advertisements. It prohibits general claims for vitamins and prescribes the units in which the quantities of the individual vitamins claimed to be present are to be declared.

1947—No. 161. The Edible Gelatin (Control) Order, 1947. Dated January 30th, 1947. Pp. 4. Price 1d.

This Order creates by definition a product known as edible gelatin and controls its use, as from February 23rd, 1947.

The following definition is given in Article 1.

"Edible gelatin" means the clean, wholesome protein which—

(*a*) is obtained by extraction from collagenous material;

(*b*) is free from objectionable taste and offensive odour when in a warm 5 per cent. aqueous solution;

(*c*) contains, when air-dried, not more than 3·25 per cent. by weight of mineral matter; and

(*d*) contains, when air-dried, in each million parts by weight, not more than 1·4 parts by weight of arsenic (expressed as arsenious oxide—As_2O_3), 10 parts by weight of lead, 30 parts by weight of copper or 100 parts by weight of zinc.

Article 2. No person shall use any gelatin other than edible gelatin in the manufacture of any food.

Article 3. No person shall use any edible gelatin in the manufacture of any food other than specified food:

Provided that nothing in this Article shall prohibit—

(*a*) the use of edible gelatin in the manufacture of any food otherwise than for sale;

(*b*) the use of edible gelatin in the manufacture of any medicine or drug, medicinal capsule, or edible container for any medicine or drug; or

(*c*) the use of any specified food containing edible gelatin in the manufacture of any other food

"Specified food" means any food specified in the Schedule to this Order, *viz.*,

Bakers' prepared materials.
Biscuits, as defined in the Biscuits (Maximum Retail Prices) Order, 1943.
Canned soup.
Confectionery, as defined in the Chocolate, Sugar Confectionery and Cocoa Products (Control and Maximum Prices) Order, 1944.
Flavouring Essences, as defined in the Flavouring Essences (Current Prices) Order, 1943.
Invalid Foods.
Meat Products, as defined in the Meat Products, Canned Soup, and Canned Meat (Control and Maximum Prices) Order, 1946.
Table Jellies, as defined in the Table Jellies (Maximum Prices) Order, 1946.
Wine and spirit finings.

"Sale," in this Order, includes also the use by a caterer for the purpose of his business.

British Standards Institution

A FEW copies of the following draft Specifications, issued for comment only, are available to interested members of the Society and may be obtained on application to the Secretary, J. H. Lane, 7-8, Idol Lane, London, E.C.3.

Draft Specifications prepared by Technical Committee OSC/24, British National Committee on Soaps and Fats of the International Commission for the Study of Fats.

CH(OSC)8024 — Draft for the Preparation of the Sample for Examination.
CH(OSC)8025 — Draft for Ash Content.
CH(OSC)8026 — Draft for Unsaponifiable Matter.
CH(OSC)8027 — Draft for Saponification Value.
CH(OSC)8028 — Draft for Free Acidity.
CH(OSC)8029 — Draft for Refractive Index.
CH(OSC)8030 — Draft for Iodine Value (Modified Wijs' Method).
CH(OSC)8031 — Draft for Hydroxyl Value or Acetyl Value.
CH(OSC)8032 — Draft for Volatile Acids (Reichert, Polenske and Kirschner Values).
CH(OSC)8033 — Draft for Impurities.
CH(OSC)8034 — Draft for Thiocyanogen Value.
CH(OSC)8035 — Draft for Volatile Matter and Water.

Draft Specifications prepared by Technical Committee OSC/12—Vegetable Oils—

CH(OSC)8535 — Draft for Crude Coconut Oil (Revision of B.S.628).

CH(OSC)8536 — Appendix I to CH(OSC)7122 — Draft for Castor Oil (First Quality) (Revision of BS.650).

Revised Draft Specification prepared by Technical Committee OSC/21 — Marine Animal and Fish Oils.

CH(OSC)8870 — Draft for Technical Compound Cod Oil.

A Draft Specification, CH(RDE)7772, Draft British Standard on Methods of Test for Soil Classification and Compaction, has been issued for comment. Copies of this Draft may be obtained from The British Standards Institution, 28, Victoria Street, London, S.W.1.

The preparation of this British Standard was undertaken by a Committee appointed by the Road Engineering Industry Committee in July, 1943, to consider the preparation of standard methods of test for stabilised soils. It consists of 24 sheets of text dealing with proximate analysis, physical testing and soil compaction tests, 13 sheets of recommended report forms and 24 diagrams of apparatus, graphs and charts; and is of interest, chiefly, to the soil chemist and civil engineer.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Determination of Free Fatty Acids in Dried Egg Powders. C. M. Johnson and L. Kline (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 617–619)—In previous work (*Ibid.*, 1946, **18**, 35; Analyst, **1946, 71,** 289) differences were reported between the total acidity of the ethereal extract of dried egg powder as determined by the method of the A.O.A.C. and the free fatty acidity of the powder, over 70 per cent. of the acidity of the extracts of some unstored powders being due to cephalin. The amount of cephalin appearing in the extract, in contrast with the free fatty acid content, was influenced by the moisture content of the powder, so that it was necessary to dry the powders uniformly as specified in the A.O.A.C. method in order to obtain reliable total acidity values. When recovery experiments were made, it was found that oleic acid which had been added to liquid egg mixtures was apparently bound in a way that prevented its extraction from the corresponding dried egg powder unless the liquid egg was adjusted to *p*H 4 before drying. Thus reliable free fatty acid values for the dried egg powders could be obtained only by reconstituting the powder, acidifying to *p*H 4 and re-drying before extraction and, in addition, a correction for cephalin was necessary.

A more satisfactory method of determining the true free fatty acid content is now described, and additional information on the combination of free fatty acids in the liquid whole egg is presented. The proposed method makes use of the differential solvent power of acetone at room temperature. Glycerides and fatty acids are soluble in cold acetone whereas the phospholipids are only slightly soluble. Traces of dissolved phospholipid can be removed by treatment with alcoholic magnesium chloride, and the phosphorus content of the treated extract is then less than 0·01 per cent.

Procedure—To 2 g. of dried egg powder in a 40-ml. graduated conical centrifuge tube, add 15 ml. of C.P. acetone and, after 30 min., centrifuge the mixture. Repeat the extraction four times, using 10-ml. portions of acetone and only 5- to 10-min. extraction periods, and stirring the suspensions frequently during the extraction periods. Combine all the extracts in a single centrifuge tube keeping the total volume below 60 ml., add, with rapid swirling, 1 ml. of absolute ethyl alcohol saturated with magnesium chloride hexahydrate, centrifuge the liquid, and transfer the supernatant portion to a 500-ml. separating funnel with the stem cut down to 1 in. Wash the precipitate twice by suspending it in 5 ml. of acetone and centrifuging, and add the washings to the liquid in the separating funnel. To the combined extracts and washings add 30 ml. of light petroleum (Skellysolve B), mix thoroughly, add 200 to 250 ml. of water, and agitate the mixture gently. The volume of acetone in the funnel should be kept as low as possible (60 to 80 ml.) because the acetone concentration in the washing procedure must lie between 20 and 30 per cent. to prevent loss of fatty acids. After separation of the phases, discard the acetone layer and wash the petroleum layer with two 25-ml. portions of water. The washings should give no reaction for chlorides. Transfer the petroleum layer to a 125-ml. glass-stoppered Erlenmeyer flask, add 50 ml. of *iso*propanol and 10 drops of 1 per cent. alcoholic phenolphthalein solution, and bubble a stream of carbon dioxide-free air through the liquid for 10 min. Titrate the solution with 0·025 *N* sodium ethylate, maintaining the stream of carbon dioxide-free air. Express the result as oleic acid, which is said to constitute 50 per cent. of the egg fatty acids (Riemenschneider *et al.*, *J. Biol. Chem.*, 1938, **126**, 255; Analyst, 1939, **64**, 48).

Recovery of oleic acid was determined by adding known amounts of oleic acid in acetone solution to samples of spray-dried egg powder suspended in acetone. These samples with controls were subjected to the procedure described with satisfactory recovery of the added oleic acid. Samples of the same egg powders were also exhaustively extracted with ether in a Soxhlet apparatus, the ether was removed by a stream of nitrogen, and the residue was dissolved in acetone and treated with magnesium chloride in the manner described. Thus only the effect of different types of extraction was studied. The results showed that the shorter acetone extraction procedure gave results from 2 to 7·5 per cent. lower, but an error of this magnitude is probably permissible for many purposes. The longer Soxhlet extraction with ether may be desirable with some samples. Since, in the proposed method, the cephalin content of the extract is negligible, preliminary drying of the sample is not necessary. As with ether, oleic acid, when added to liquid egg before drying, was incompletely recovered by neutral acetone. Therefore a modified procedure based upon the use of acidified acetone (0·088 *N* with respect to hydrochloric acid) was used with samples to which oleic acid had been added before drying. The acidified acetone was used in the first two extractions and neutral acetone in the remaining three, because high results due to extraction of phosphorus compounds that were not removed by magnesium chloride treatment were obtained when acidified acetone was used in the last three extractions. Although the pH of the suspensions of the residues from the acid acetone extraction was near 4, only 80 to 90 per cent. recovery could be attained by the method, and it is not recommended as a means of obtaining complete extraction of fatty acids from egg samples that may contain bound fatty acids, and the procedure involving reconstitution, adjusting to pH 4, and lyophilising is recommended for these. The fatty acids in the dried adjusted powder may then be determined by the proposed method.

Although recovery of oleic acid added to, or present in, liquid egg mixtures before drying is not complete unless the pH of the liquid is adjusted to 4, extraction is complete from egg powders which have developed free fatty acidity during storage in the dry state, and the recovery is not increased by the adjustment of the acidity of either spray-dried or lyophilised powders to pH 4. If, on the other hand, samples of egg powders in which acidity has developed are reconstituted and allowed to stand in the liquid state at its natural pH for a time before being lyophilised, then part of the fatty acids is no longer extractable unless the pH is adjusted to 4. These results may be explained by assuming that liberation of fatty acids in the powder occurs in a substantially fatty medium, *i.e.*, the fatty acids are, in a microscopic sense, near the egg lipids, and, for this reason, or because the medium is essentially non-aqueous, the fatty acids have little opportunity to combine with bases. When fatty acids occur in the liquid egg they are free to combine with the bases to an extent determined by the pH of the mixture. Experiments showed that, as might be expected, egg white binds fatty acids more readily than egg yolk. The proteins of the white contain many basic groups, and lysozyme, an unusually basic protein constituent of egg white, is known to form salts with strong acids and, doubtless, would react similarly with the higher fatty acids.

The lowest moisture content at which interaction of the fatty acids and proteins occurs is not known. However, egg powders containing as much as 8 per cent. of moisture gave the same free fatty acid values before and after adjustment of the pH. Since few dried egg powders are prepared at higher moisture levels, no adjustment of the pH of dried egg powders prepared from good shell eggs seems necessary.

The production of lactic acid in certain types of egg deterioration has been reported (Brookes and Hawthorne, *J. Soc. Chem. Ind.*, 1943, **62**, 181), but, since lactic acid is soluble in water, it is not determined by the procedure outlined. The method would not be applicable to material likely to contain fatty acids that are water-soluble, since they would be incompletely partitioned between the aqueous acetone and the petroleum layers in the washing process, thus leading to low results. Since the lower fatty acids are absent from egg fat (Riemenschneider *et al., loc. cit.*), such losses do not occur in the determination of egg fatty acids arising from the usual type of glyceride hydrolysis. Moderate excess of acids added to lower the pH of the sample do not interfere in the proposed method, since they are removed during the washing process. A. O. J.

Iodimetric Method for the Assay of Penicillin Preparations. J. F. Alicino (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 619–220)—Penicillin is inert to iodine in neutral aqueous solution, whereas penicilloic acid, formed by inactivation of penicillin by means of alkali or penicillinase, consumes from 6 to 9 equivalents of iodine, depending on the conditions used. The difference between the iodine consumed under standard conditions before and after inactivation by alkali is, with certain limitations, proportional to the penicillin content.

Procedure—Dissolve 3 to 5 mg. of crystalline sodium penicillin II (G) in 5 to 10 ml. of water and add 0·5 ml. of N sodium hydroxide. Leave for 15 min. and then neutralise with 0·5 ml. of N hydrochloric acid. Add a measured excess (*e.g.*, 10 ml.) of 0·01 N iodine and, after 30 min., titrate with 0·01 N sodium thiosulphate. Under these conditions, 1 mg. of sodium penicillin II (G) consumes 2·52 ml. of 0·01 N iodine, corresponding to 8·97 g. equivalents of iodine per g-mol. Sodium penicillin I (F) under the same conditions consumed 2·64 ml. per mg. or 8·8 g.-equivalents per g.-mol. Penicillin III (X) may give abnormally high values

In the assay of an unknown, inactivate in a similar manner a solution containing 1000 to 5000 units, add iodine, and back-titrate as described above. Carry out a blank titration in which the inactivation is omitted. For relatively pure preparations (800 to 1000 μg. per mg.) the difference between the two values is proportional to the penicillin content, but crude preparations give results considerably lower than the bioassay values. The discrepancy between iodometric and bioassay values can be reduced by back-titrating the blank solution immediately after addition of the iodine solution, although the alkali-inactivated sample is allowed to stand for 30 min. before titration with the thiosulphate solution.

With this modification, the method gave results in satisfactory agreement with the bioassay values for samples containing from 10 to 97 per cent. of penicillin. F. A. R.

Biochemical

Adsorption of Aneurine on Sand. II. N. Ridyard (*J. Soc. Chem. Ind.*, 1946, **65**, 92–95)—In the course of an investigation of synthetic zeolites it was found that a control column of acid-washed sand completely removed aneurine from an acid aqueous solution, and that the vitamin could be recovered from the sand by elution with saturated potassium chloride solution. Sand was in fact found to be a very convenient adsorbent for aneurine, the best results being obtained with a china-clay sand from St. Austell and with a sand from King's Lynn (Messrs. Boam). The Norfolk sand proved to be the more homogeneous, and was selected for use in the estimation of aneurine in wheat products.

Procedure—Wash the sand about six times with 0·2 N hydrochloric acid to remove dirt and fine material, and transfer the acid slurry into a column, 220 mm. long and 15 mm. in diameter. Allow a few ml. of acid to run through the column and then pour 10 ml. of the extract (10 g. of flour or 5 g. of wholemeal or bran per 50 ml.) on to the column. Allow to drain and wash with two 2-ml. portions, followed by about 20 ml., of 0·2 N hydrochloric acid. Pour 10 ml. of a saturated potassium chloride solution in 0·2 N hydrochloric acid on to the column and allow to drain. Wash the column with 2 or 3 portions of acid water until 2·5 ± 0·2 ml. have collected in a graduated tube placed below the column. Replace this tube by a second tube and continue washing until 13 to 14 ml. have collected. Collect another 3 to 4 ml. in the first tube and retain this solution until it has been confirmed that the whole of the aneurine has collected in the second tube. Estimate the aneurine in this solution by the thiochrome method, using a standard solution containing the same concentration of salt as the eluate; the presence of the potassium chloride in the eluate affects the partition of the thiochrome between the *iso*butanol and the aqueous layer. The aneurine content should not be calculated from the result obtained with a standard solution that has been passed through a 220 mm. sand column, as only 90 per cent. of the aneurine is recovered on subsequent elution, whereas 100 per cent. is recovered from flour extracts under the same conditions; the difference is due to the presence in the flour extract of adsorbable material other than aneurine. The most suitable standard is prepared by saturating 10 ml. of a standard solution with potassium chloride and diluting to 13 ml. This is used in addition to the usual four standard tubes in each run to fix the extinction-concentration curve. F. A. R.

Microbiological Estimation of Methionine in Proteins and Foodstuffs. W. H. Riesen, B. S. Schweigert, and C. A. Elvehjem (*J. Biol. Chem.*, 1946, **165**, 347-358)—The requirements of methionine by the three organisms *Lactobacillus arabinosus*, *Streptococcus faecalis*, and *Leuconostoc mesenteroides* have been investigated. The following basal media were used:

Constituents	*Lactobacillus arabinosus* (Schweigert *et al.*, *J. Biol. Chem.*, 1944, **155**, 183) mg. per tube	*Streptococcus faecalis* (Greenhut *et al.*, *J. Biol. Chem.*, 1946, **162**, 69) mg. per tube	*Leuconostoc mesenteroides* (Dunn *et al.*, *J. Biol. Chem.*, 1944, **156**, 703) mg. per tube
Amino acids			
Oxidised casein hydrolysate	20 (0·2 ml.)	50 (0·5 ml.)	50 (0·5 ml.)
l(+)-Glutamic acid	4	4	4
l-Asparagine	4	4	4
l(+)-Lysine monohydrochloride+H_2O	2	2	2
dl-Threonine	2	2	2
dl-Valine	2	2	2
dl-*Iso*leucine	2	2	2
dl-Alanine	2	1	2
l(−)-Cystine	1	2	2
dl-Leucine	2	2	2
dl-Phenylalanine	1	1	1
l(+)-Arginine hydrochloride	0·5	0·5	1
l(+)-Histidine hydrochloride+H_2O	0·5	0·5	1
l(−)-Tyrosine	0·5	1	1
dl-Tryptophan	0·5	1	1
Glycine	—	0·2	1
dl-Serine	—	0·5	2
l(−)-Proline	—	—	0·5
Glucose	200	200	200
Sodium acetate	200	—	200
,, citrate+H_2O	—	250	—
Purines and pyrimidines	(0·1 ml.)	Same as *L. arabinosus*	Same as *L. arabinosus*
Adenine sulphate+$2H_2O$	0·1	,, ,,	,, ,,
Guanine hydrochloride+$2H_2O$	0·1	,, ,,	,, ,,
Uracil	0·1	,, ,,	,, ,,
Xanthine	—	0·1 (0·1 ml.)	0·1 (0·1 ml.)
Salts A	(0·05 ml.)	—	Same as *L. arabinosus*
KH_2PO_4	5	—	,, ,,
K_2HPO_4	5	50 (0·1 ml.)	,, ,,
Salts B	(0·05 ml.)	Same as *L. arabinosus*	,, ,,
$MgSO_4.7H_2O$	2	,, ,,	,, ,,
NaCl	0·1	,, ,,	,, ,,
$FeSO_4.7H_2O$	0·1	,, ,,	,, ,,
$MnSO_4.4H_2O$	0·1	,, ,,	,, ,,
	µg. per tube	µg. per tube	µg. per tube
B-vitamins	(0·1 ml.)	,, ,,	,, ,,
Aneurine hydrochloride	5	,, ,,	,, ,,
Riboflavine	5	,, ,,	,, ,,
Nicotinic acid	10	,, ,,	,, ,,
dl-Calcium pantothenate	5	,, ,,	,, ,,
Pyridoxamine dihydrochloride	12	,, ,,	,, ,,
Pyridoxine hydrochloride	50	,, ,,	,, ,,
p-Aminobenzoic acid	5	,, ,,	,, ,,
Biotin	0·01	,, ,,	,, ,,
Folic acid	0·1	,, ,,	,, ,,
Choline chloride	25	,, ,,	,, ,,
i-Inositol	25	,, ,,	,, ,,

Stock solutions were prepared in the concentrations indicated above. Several millilitres of hydrochloric and sulphuric acid were added to Salts B to prevent precipitation. The purines and pyrimidines were dissolved in hot hydrochloric acid. Xanthine was dissolved in aqueous ammonia. The B vitamins were dissolved by heating in water. New vitamin solutions were prepared every month. Convenient volumes for all solutions were 500 ml. They were stored under toluene in a refrigerator. The amino acids were ground with a mortar and pestle, and dissolved in a few millilitres of dilute hydrochloric acid prior to each assay. Oxidised casein hydrolysate was prepared by the method of Toennies (*J. Biol. Chem.*, 1942, **145**, 667), which is as follows:

Dissolve 400 g. of casein in 2·4 litres of formic acid, and rapidly add 400 ml. of 30 per cent. hydrogen peroxide with stirring. After 2–3 mins., cease stirring and leave for 1 hour. Add 10 litres of water and add, with stirring and cooling, ammonia to *p*H 4·0 to 4·5; the temperature should not exceed 35° C. Filter and re-suspend the solid in 15 litres of water. Again filter, re-suspend in water, filter, and suspend in 12 litres of methanol; allow to settle overnight, filter, and again suspend in methanol. After standing overnight, filter, and dry the solid in a desiccator and then in a vacuum drying oven at 70° C.

The standard curves obtained with the three organisms indicated that all could be used for the assay of methionine. The maximum growth obtained with *Lactobacillus arabinosus* was greater than that obtained with the other two organisms, whilst smaller amounts of methionine resulted in a greater growth response with this organism than with the others. A suitable assay range with *L. arabinosus* was 0 to 20 μg. of *l*-methionine per tube as compared with 0 to 50 μg. for the other two organisms. Good recoveries of methionine added prior to the hydrolysis of proteins (using 2 *N* hydrochloric acid at 15 lbs. pressure for 5 to 10 hours) were obtained, and the methionine contents of the proteins analysed agreed satisfactorily with the values recorded in the literature. F. A. R.

Use of Hydrogen Peroxide-treated Peptone in Media for the Microbiological Determination of Amino Acids. C. M. Lyman, O. Moseley, S. Wood, and F. Hale (*Arch. Biochem.*, 1946, **10**, 427–431)—Media for the micro-biological estimation of amino acids can be prepared more cheaply by preferential destruction or removal of certain amino acids from protein than by the use of pure amino acids. A method of destroying methionine, cystine, tryptophan, and tyrosine in peptone by treatment with hydrogen peroxide is described (*cf.* Toennies, *J. Biol. Chem.*, 1942, **145**, 667). Smooth and regular standard curves for methionine, tryptophan, and tyrosine were obtained with media in which most of the amino acid nitrogen was supplied by hydrogen peroxide-treated peptone.

Method—Treat 50 g. of Bacto-peptone in 500 ml. of *N*-hydrochloric acid with 0·05 g.-mol. of hydrogen peroxide (5·7 g. of 30 per cent. hydrogen peroxide) and leave overnight at room temperature. Heat the solution in a steam steriliser at atmospheric pressure for 30 mins., cool, neutralise with sodium hydroxide, and steam again for 1 hr. After cooling, dilute to 1 litre.

The medium used for tryptophan is prepared by replacing the amino acids in the medium of Kuiken *et al.* (*J. Biol. Chem.*, 1943, **151**, 615; ANALYST, 1944, **69**, 156) by 50 mg. of hydrogen peroxide-treated peptone supplemented by 1 mg. each of methionine, cystine, and tyrosine per 10 ml. The medium used for tyrosine is prepared by replacing the amino acids in the medium of McMahan and Snell (*J. Biol. Chem.*, 1944, **152**, 83; ANALYST, 1944, **69**, 188) by 50 mg. of the treated peptone, supplemented by 0·5 mg. of tryptophan and 1 mg. each of methionine and cystine per 10 ml. The medium used for methionine is prepared by replacing the amino acid mixture in medium D of Dunn *et al.* (*J. Biol. Chem.*, 1944, **156**, 703; ANALYST, 1945, **70**, 182) by 75 mg. of the treated peptone, supplemented by 0·5 mg. of tryptophan and 1 mg. each of cystine and tyrosine per 10 ml. F. A. R.

Colorimetric Method for the Estimation of Vitamin D, based on Formation of Carbenium Salts. H. Schaltegger (*Helv. Chim. Acta*, 1946, **29**, 285–302)—Ergosterol and calciferol give characteristic colours with aromatic aldehydes in the presence of concentrated sulphuric acid, owing to the formation of carbenium sulphates, the sterol in the ionised state behaving as a weak base; different aldehydes produce different colours. Perchloric acid can be used in place of sulphuric acid, with benzene and glacial acetic acid as solvents. Ionising solvents, such as alcohols, should be absent. The colours formed with certain aldehydes are quite stable and can be used for the estimation not only of ergosterol and calciferol, but also of cholesterol and other sterol derivatives, the colours being evaluated at appropriate wavelengths. Three types of reaction can be distinguished, according to the number of ionisable carbon atoms present in the molecule:

Type 1—All sterols containing, besides the one ionisable ethylene group, no other double bonds or only double bonds not in conjugation with a co-ordinate linkage, give only small quantities of carbenium salt and thus give only feeble colours.

Type 2—All sterols containing conjugated double bonds with two ionisable carbon atoms, *e.g.*, ergosterol and 7-dehydrocholesterol, yield considerable amounts of carbenium salts, giving more intense colours than compounds of Type 1.

Type 3—All the D vitamins and tachysterols containing three conjugated double bonds in a chain give carbenium salts with colours 10 times as intense as the salt derived from ergosterol.

Bile acids give feeble colours with aromatic aldehydes and do not interfere with the estimation of vitamin D. β-Carotene and vitamin A, however, give colours similar to those obtained with vitamin D. Aneurine, riboflavine, ascorbic acid, tocopherol, vitamin K, and linoleic acid do not give colours with the reagent. For the estimation of vitamin D, three aldehydes proved to be particularly suitable namely, anisaldehyde, vanillin, and 4-hydroxy-1-naphthaldehyde, which give bluish-violet, blue, and green colours, respectively.

Procedure—For the estimation of vitamin D in irradiated ergosterol or irradiated 7-dehydrocholesterol, remove unchanged ergosterol by dissolving in alcohol, cooling to 0° C., and filtering off the crystalline solid. Evaporate to dryness a portion of the filtrate containing 0·2 to 0·3 g. of dry matter and leave in a desiccator for 2 hr. Dissolve the residue in benzene and make up to a volume of 20 ml. To 2 ml. of this solution add from 0·1 to 0·15 ml. of acetic anhydride and 5 ml. of benzene, and heat for half an hour at 75° C. Destroy the tachysterol in the acetylated mixture by adding 10 to 15 mg. of freshly distilled maleic anhydride and 5 ml. of benzene and then warming for 20 to 30 min. at 75° C. Cool the solution, filter off the adduct and dilute the filtrate to 20 ml. with benzene. Dilute this solution 10-fold and mix 1 ml. of the dilute solution with 1 ml. of a 0·1 per cent. solution of 4-hydroxy-1-naphthaldehyde in benzene and 1·5 ml. of benzene (purified by being heated under reflux for 2 hr. with anhydrous aluminium chloride, distilled, washed with water, dried with sodium sulphate, and re-distilled). Heat to boiling and add 2 drops of perchloric acid reagent (mix 2 ml. of acetic anhydride, 2·5 ml. of glacial acetic acid and 0·5 ml. of 70 per cent. perchloric acid, and heat under reflux for half an hour in an oil-bath at 95 to 100° C. Use a pipette that delivers a drop weighing approximately 20 mg.) Heat for a further minute and then allow to cool to room temperature. Add 1·5 ml. of glacial acetic acid and measure the extinction value (K) of a 1-cm. layer using filter S.66. The amount of vitamin D is equivalent to $150\,K + 10$. The corresponding values when anisaldehyde and vanillin are used are $62{\cdot}1\,K + 2{\cdot}5$ and $106{\cdot}5\,K + 2{\cdot}75$, respectively. These aldehydes are also employed in the form of 0·1 per cent. solutions in benzene, but filter S.57 is used instead of S.66. This estimation gives the approximate amount of vitamin D present. To obtain a more accurate value, repeat the procedure using a volume of the final benzene solution such that 50 to 80 μg. of vitamin D are present. Mix this with 1 ml. of the anisaldehyde reagent and dilute with benzene to 3·5 ml. Heat to boiling, add 2 drops of the perchloric acid reagent, heat for a further minute, and allow to cool. Add 1·5 ml. of glacial acetic acid and evaluate the colour as before.

To estimate tachysterol repeat the estimation, omitting the maleic anhydride treatment, and from the result subtract the value previously obtained.

Vitamin D in fish oils containing at least 500,000 I.U. (12·5 mg.) per ml. can be estimated directly without saponification, but a blank estimation must be carried out on the oil. Oils containing less than 500,000 I.U. per ml. must first be saponified, and the reaction applied to the non-saponifiable matter.

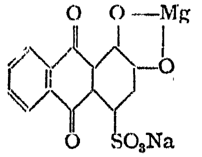
The results obtained with solutions of pure vitamins D_2 or D_3 seldom differed from the theoretical by more than 5 per cent. F. A. R.

Colorimetric Method for the Estimation of Reducing Steroids. R. D. H. Heard and H. Sobel (*J. Biol. Chem.*, 1946, **165**, 687–698)—The reduction of phosphomolybdic acid to molybdenum blue has been applied to the quantitative estimation of small amounts (10 to 100 μg.) of reducing steroids related to the adrenal cortex hormone. The reaction is given by steroids containing a primary or secondary, but not tertiary, α-ketol function, or an αβ-unsaturated 3-keto group, or both.

Procedure—Put 0·1 ml. of a solution of the steroid in glacial acetic acid, containing the equivalent of about 100 μg. of desoxycorticosterone, into a small test tube and add 2·00 ml. of phosphomolybdic acid reagent prepared by mixing equal volumes of glacial acetic acid and the phosphomolybdic acid solution of Folin and Wu (*J. Biol. Chem.*, 1920, **41**, 367). Immerse the tube in a constant-volume bath of boiling water for exactly 60 min., cool immediately, and transfer the contents of the tube quantitatively with 8·00 ml. of the reagent to one of a pair of optically matched Evelyn colorimeter tubes. After allowing air bubbles to rise to the surface (about 90 sec.), read within the next 4 min. the optical density at 650 to 660 mμ. Run a blank estimation simultaneously in the same way with 0·10 ml. of glacial acetic acid and 2·00 ml. of reagent, and use the solution obtained to adjust the colorimeter to zero optical density. The results are calculated from a standard curve prepared with the aid of pure desoxycorticosterone. F. A. R.

Detection of Alkaline Earth Metals in the Cell Tissue of Plants. R. Haller (*Helv. Chim. Acta*, 1946, **29**, 8–11)—In a study of the reactions of the mucilage of the endosperm of *Ceratonia siliqua* (Carob bean) it was noticed that the sodium salt of alizarin monosulphonic acid (alizarin red SW or alizarin S), although not staining the mucilage, gave a characteristic red colour to certain cell contents. Formation of this colour is not entirely due to protein since protein yields a brown colour and not the intense red colour of this reaction. Development of the red colour indicates the presence of metallic salts forming complex compounds with alizarin. Calcium and barium compounds form vivid blue-violet lakes, and aluminium and magnesium compounds form red lakes. Aluminium is found only exceptionally in a few domestic Lycopodium species and in certain tropical plants, and, as far as the author knows, barium salts do not occur in plants. [*Abstractor's note*: Seaber, ANALYST 1933, **58**, 575, reports the occurrence of barium in Brazil nuts.] Magnesium, however, is an essential constituent of chlorophyll, and it seemed probable that magnesium salts were responsible for the formation of the red colour.

To determine if the colour can be observed in fresh cell tissue, experiments were made with sections of the leaf stalk of *Cucurbita pepo*, L. When sections are placed in a solution of alizarin monosulphonic acid and observed microscopically, a vivid scarlet colour appears after a short time especially near the vascular bundles and in the cell contents of the parenchyma. Gradually the mounting liquid is coloured red by escape of the cell contents from the tissue. The epidermis, however, with the exception of the outermost layer (the true cuticle), remains uncoloured. The cuticle is coloured intensely red, as are the tissues in the neighbourhood of the vascular bundles, and this is true also of the tissue of *Urtica dioica* L. and *Heracleum spondylum* L. Apparently, the magnesium compounds occur in the cell sap and are transported thence for the synthesis of chlorophyll. Tissues containing magnesium entirely in the form of chlorophyll do not give the reaction. If the leaf stalk of *C. pepo* is hardened in alcohol and sections are subjected to the reaction, very little colour develops, but if the hardening alcohol is treated with sodium alizarin monosulphonate a vivid red precipitate is thrown out, so that evidently the magnesium salt is extracted from the tissue. When sections of the stem of *Orobranche lucorum* L. (a parasite on *Berberis* spp.), a plant containing no chlorophyll, are placed in an aqueous solution of sodium alizarin monosulphonate and examined microscopically, no trace of colour is evident and, as might be expected, no magnesium compounds occur in the cell sap of this plant If pieces of the stem of *C. pepo* are placed in alcohol for 24 hr. and the pale green alcoholic solution is treated with alcoholic sodium alizarin monosulphonate, a red precipitate forms indicating solubility of the magnesium compound in alcohol. This red precipitate is soluble in water forming a red solution, and it may be the compound



Its neutral aqueous solution dyes wool an intense purple red, a colour quite different from that formed with sodium alizarin sulphonate alone. When sections of the stem of *C. pepo* are mounted in silver nitrate solution and examined microscopically, a precipitate darkened by the action of daylight, and soluble in ammonia, forms in the cell sap, indicating that magnesium is present as chloride, which is appreciably soluble in alcohol. The presence of magnesium can be demonstrated by extracting the ignited red precipitate with diluted sulphuric acid and separating magnesium as magnesium ammonium phosphate; the sensitive test of Hahn with quinalizarin (tetraoxy-anthraquinone) can also be applied, and calcium does not interfere with this test. The reagent is insoluble in water, but yields a blue-violet solution when *N* alkali is added to its aqueous suspension. When a solution of a magnesium compound is added to this solution a pure pale blue colour develops and very soon a light blue precipitate forms even in very dilute solutions. The reaction is not applicable to plant sections. The cell walls are coloured a very pale blue, but the characteristic blue precipitate cannot be obtained, possibly owing to the protective colloid action of proteins.

Two East Indian plants, *viz.*, *Symplocos fasciculata* Zoll. and *S. spicata* Roxb., are used by the natives in the dyeing of cotton fabric. Fragments of the leaves of these plants, when immersed in alizarin red SW solution gave a reddish granular precipitate due to presence of aluminium compounds. Magnesium was found to be present in the ash of the leaves of *S. spicata*, but apparently plays no part in the formation of the red lake. When every trace of calcium was removed from cotton fabric and the dyeing process was carried out in a mordant solution containing aluminium and magnesium acetates, the colour produced was an unstable pale rose. In presence of calcium salts, however, an intense fast red colour was imparted to the fabric. A. O. J.

Organic

Indicator Properties of Derivatives of 4′-Nitrophenylazo-1-naphthol.

K. H. Ferber (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 631–632).—Three new indicators of the 2-(4′-nitrophenylazo)-1-naphthol class, changing from a red neutral form to a blue alkaline form over various *p*H ranges, are described.

Preparation—I. *Disodium salt of* 2-(4′-*nitrophenylazo*)-1-*naphthol*-4:8-*disulphonic acid. Alpha blue*—To a stirred mixture of 1000 g. ice, 250 ml. of hydrochloric acid (20° Bé), and 2 g. of 95 per cent. sodium nitrite, add a mixture of 138 g. of *p*-nitraniline and 72 g. of sodium nitrite in 1 litre of water. After 2 hrs., filter and add the filtrate slowly to a mixture of 1 g.-mol. of 1-naphthol-4:8-disulphonic acid, 370 g. of sodium carbonate, 1000 g. of ice, and 1·5 l. of water. Agitate overnight, filter, re-slurry in 3 l. of 10 per cent. sodium chloride solution, filter, and dry at 60° C. The reported purity of the product, determined by titration with titanous sulphate, is 67·1 per cent. coal-tar dye. II. *Disodium salt of* 2-(4′-*nitrophenylazo*)-1-*naphthol*-3:8-*disulphonic acid. Epsilon blue.*—Diazotise 1 g.-mol. of *p*-nitraniline as above, and then add the product slowly to a mixture of 1 g.-mol. of 1 naphthol-3:8-disulphonic acid, 336 g. of sodium carbonate, 1000 g. of ice, and 2 l. of water. Isolate and dry the dye as above. The reported purity of the product is 93·7 per cent. III. *Disodium salt of* 2-(2′-*methoxy*-4′-*nitrophenylazo*)-1-*naphthol*-4 : 8-*disulphonic acid. Nitroanisole blue*—Add slowly to a mixture of 168 g. of 5-nitro-2-aminoanisole, 250 ml. of hydrochloric acid (20° Bé), and 1000 g. of ice, a solution of 74 g. of 95 per cent. sodium nitrite in 150 ml. of water. After 2 hrs.' agitation, filter and couple with 1-naphthol-4:8-disulphonic acid by the method described above for Alpha blue. The reported purity of the product is 74·1 per cent. As the products contain large amounts of inorganic salts, further purification is necessary if they are to be used in unbuffered media.

Spectral transmission curves of solutions, buffered to various *p*H values and containing 10 p.p.m. of the dye, are given for each of the indicators. The presence of boric acid in Clark and Lubs' buffers for *p*H 9 and 10 was found to interfere with the dissolved dye. The colours of the solutions are: Alpha blue, at *p*H 8, pink; *p*H 9, purplish pink; and *p*H 10 to 13, light purple. Epsilon blue, *p*H 8 to 11, pink; *p*H 12, purplish pink; and *p*H 13, light purple. Nitroanisole blue, *p*H 8 and 9, pink; *p*H 10, purplish pink; and *p*H 11 to 13, light purple. Solutions of all three dyes in water, alcohol, *N* hydrochloric acid, 5 per cent. acetic acid solution, 5 per cent. sodium bicarbonate solution, and 5 per cent. solution of $Na_2HPO_4.12H_2O$ are pink. Alpha blue and Epsilon blue are light purple with 5 per cent. sodium carbonate solution, and 0·1 *N* sodium hydroxide. Epsilon blue is pale bluish purple with a 5 per cent. solution of $Na_3PO_4.12H_2O$. Alpha blue is useful for distinguishing between carbonate and bicarbonate, and Epsilon blue is recommended for controlling the alkalinity of di- and tri-sodium phosphate mixtures. Dilute aqueous solutions are only slightly affected by storage for one month in diffused daylight. To make test papers, immerse strips of filter paper in a solution of 4 g. of the dye in one litre of water, and allow to dry. The colours obtained when various reagents are spotted on such test papers are listed in terms of the matching chips from the Munsell *Book of Colour*. B. A.

Comparative Estimations of Carbonyl and Carboxyl Groups in Chromium Trioxide and Hypochlorous Acid Oxycelluloses and Oxyxylans.

B. Meesook and **C. B. Purves** (*Paper Trade J.*, 1946, **123**, Oct. 31, *T.A.P.P.I. Sect.*, 223–230)—(*a*) *Calcium Acetate Method* (*cf.* Yackel and Kenyon, *J. Amer. Chem. Soc.*, 1942, **121**, 64)—This depends on the titration with alkali of the acetic acid displaced from calcium acetate. Prepare a 0·5 *N* solution (*p*H 7·2 to 7·5) of the calcium acetate in boiling water, and filter it. Immerse *x* (0·2 to 0·5) g. of the sample in a 60-ml. aliquot portion of the solution, and after the requisite time (*e.g.*, 24 hr., *vide infra*), filter it, and titrate a 50-ml. aliquot portion of the filtrate electrometrically to *p*H 8·3 with 0·01 *N* sodium hydroxide. If, during the experiment, the *p*H falls to below 6·3, low results may be expected; then, repeat the experiment using a smaller weight of sample, or in presence of a known volume of standard alkali, which is added 1 hr. after the immersion of the sample, and which is less than the amount required to bring the *p*H to above 7·0. If y ml. of *N* alkali is the difference between the titres obtained with the sample and with a "blank," then the carboxyl content is $6y/5000x$ g.-mols. per g. (*b*) *Silver o-Nitrophenolate Method*—Warm 10 g. of *o*-nitrophenol with 500 ml. of water to 70° C., add 15 g. of silver oxide, with stirring, and after 2 hr. filter from the excess of silver oxide. Allow the solution to cool, filter off the orange-coloured prisms, dry them, and store them in a brown bottle. Warm the filtrate with 2 to 3 g. of silver oxide, filter, dilute the filtrate to 1·5 litres, and store it in a brown bottle. This solution is the reagent (*p*H 7·3); further supplies are made by dissolving 1 g. of the crystals in 1 litre of warm water. Standardise 25-ml. portions of the solution by titration with 0·04 *N* ammonium thiocyanate in presence of 5 ml. of 6 *N* nitric acid (free from nitrous acid), and 1 ml. of saturated ferric ammonium sulphate solution as internal indicator; the silver nitrophenolate solution is 0·00495 *N*, and remains so for about 6 weeks. Immerse x gram (*e.g.*, 0·5 g.) of the sample in 100 ml. of the reagent at 20° C. for about 72 hr., and titrate 25-ml. portions of the resulting solution; if the reduction in the titration as compared with the standard is y ml. of *N* thiocyanate solution, then the carboxyl content is $4y/1000x$ g.-mol. per g. (*c*) *Methylhydroxylamine Hydrochloride Method*—Dissolve 7·0 g. of the pure methylhydroxylamine hydrochloride in a mixture of 455 ml. of water and 91 ml. of 0·49 *N* sodium hydroxide (*p*H 4·6). Immerse x g. of the sample (*i.e.*, approx. 2 g. of oxycellulose, or 0·2 g. of reducing sugars) in 60 ml. of the solution for about 24 hr., remove the solid matter on a dry filter, and titrate a suitable aliquot portion (*e.g.*, 50 ml.) of the filtrate to *p*H 3·5 electrometrically with 0·1 *N* hydrochloric acid; adjust the volume with distilled water to that used in a blank titration previously made, and complete the titration to *p*H 3·0. Then the carbonyl content equals $6y/5000x$ g.-mol. per g., where y ml. of *N* acid is the difference between the titrations obtained with the sample and with the blank. If the determination is carried out on the neutral calcium oxycellulose salt formed in Method (*a*), then the result gives carbonyl only; with acidic samples, however, carbonyl plus carboxyl are given. With all of these methods the results above can be converted to g. mol. per g. mol. of sample by multiplying by 162 for oxycellulose, or 132 for oxyxylan. Methods (*a*) and (*b*) require 1 and 3 days respectively for completion at 20° C., and under these conditions the results agree to within ±3 per cent. The sharp end-point of (*b*) makes it preferable when the number of acidic groups is small, but (*a*) is preferable for (*e.g.*, hypochlorite) oxycelluloses which contain reducing groups, since with (*b*), metallic silver is sometimes produced by reduction. The reaction of Method (*c*) is approximately 95 per cent. complete in 1 day at 20° C., and the results then agree with those obtained in 1·5 hr. with hydroxylamine hydrochloride (*cf.* Gladding and Purves, *T.A.P.P.I. Papers*, 1943, **26**, 119) to within ±3 per cent.; apart from the consideration of rapidity, (*c*) is, however, preferable to the latter method in that the reagent is stable for days instead of only for hours. Applications of the methods to the study of the oxidation of cellulose by various reagents are described. J. G.

Picric Acid Method for Determination of Aromatic Content of Aviation Gasoline. C. M. Gambrill and J. B. Martin (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 689–692)—The method of Dobryanskiĭ and Tikhonov-Dubrovskiĭ (Azerbaodzhanskoe Neftyanoe Khoz., 1935, No. 5, 84) utilised the difference in solubility of picric acid in aromatic and non-aromatic hydrocarbons to determine the aromatic content of aviation gasoline; it has been developed to a more precise and rapid form, accurate to within ±1 per cent. in the range up to 25 per cent of aromatics.

Method—60 ml. of the gasoline are measured into a 125-ml. Squibb-type separatory funnel containing 1 g. of pure picric acid, preferably dry, but in no case containing over 10 per cent. of water. The mixture is brought to between 20 and 30° C. and shaken for 5 min. mechanically or by hand; if shaken by hand, the vessel is held by the finger tips to prevent change in temperature. The temperature is recorded, and the liquid is filtered through a Whatman No. 1 or other fast paper into a 125-ml. conical flask. A 50-ml. sample of the filtrate is pipetted to a 250-ml. flask, 3 drops of *m*-cresol purple indicator are added, and the liquid is titrated with 0·05 *N* alkali until it becomes purple; a 1-ml. excess of alkali is added. The mixture is then shaken for 1 minute vigorously, the two layers are allowed to separate, and the excess of alkali is titrated with 0·01 *N* hydrochloric acid to the complete disappearance of the reddish-purple colour as viewed under a tungsten filament lamp. The number of mg. of picric acid per 100-ml. of the saturated solution = 458·2 × (ml. of *N* sodium hydroxide − ml. of *N* hydrochloric acid). This is corrected to solubility at 25° C. by multiplying it by $[1 + 0{\cdot}035\,(25-t)]$, where t is the temperature of determination. Percentage of aromatics is read off from a curve drawn from the following data:

Percentage of aromatics	Solubility of picric acid at 25° C. (mg. per 100 ml.)
2	18·5
5	32·8
10	67·3
15	119·8
25	297·8

The mixture of aromatics used in obtaining these figures was one of benzene : toluene : xylene, 1:3:2, which the authors claim to give a good average representation of most aviation gasoline. The presence of olefins and naphthenes necessitates corrections of −1·4 per cent. for each 10 per cent. of olefins and −0·6 per cent. for each 10 per cent. of naphthenes. Reproducibility is about ±0·2 per cent.

Determination of some Aromatic Amines and Substituted Ureas in Smokeless Powder. Improved Volumetric Bromination Procedure. T. D. Waugh, G. Harbottle, and H. M. Noyes (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 636–637)—For the quantitative determination of stabilisers in smokeless powders a volumetric bromination procedure has been widely used. This involves treatment of a solution of the powder extract with a known amount of standard bromate-bromide solution, acidification to liberate bromine, addition of iodide at the end of the bromination period, and titration of the liberated iodine with standard sodium thiosulphate solution. This method has been used in the determination of diphenylamine, ethyl centralite (N,N'-diethyl-N,N'-diphenylurea), and acardite (N,N'-diphenylurea). Under the conditions of the procedure, 1 molecule of diphenylamine reacts with 4 molecules of bromine, and 1 molecule of ethyl centralite, or acardite reacts with 2 molecules of bromine. In the procedure developed for centralite by Levenson (*Ibid.*, 1930, **2**, 246), the bromination and titration are carried out in a one-phase system in presence of alcohol to keep the stabiliser in solution under carefully controlled conditions of temperature and time in order to obtain quantitative bromination of the stabiliser without side reactions between the bromine and the alcohol. To avoid this precise control, Ellington and Beard (*J. Soc. Chem. Ind.*, 1931, **50**, 151T) used carbon tetrachloride as solvent, and carried out the bromination and titration in the resulting two-phase system. It is now found that the advantages of both procedures may be obtained by the use of glacial acetic acid as the solvent. In most procedures for the estimation of stabilisers in smokeless powder the sample is extracted directly with ether, or is decomposed by alkali, distilled with steam, and the distillate extracted with ether. When the powder was prepared by either of these procedures for the carbon tetrachloride bromination method, results were low by as much as 10 per cent. Subsequent investigation showed this to be due to peroxides in the ether, and the replacement of ether by methylene chloride as extracting solvent led to satisfactory results.

Procedure—Place the finely divided powder containing not more than 0·075 g. of ethyl centralite, 0·02 g. of diphenylamine, or 0·06 g. of acardite into a Soxhlet extractor attached to a 250-ml., glass-stoppered iodination flask containing 100 ml. of methylene chloride. Extract the sample for 2 hr. or more, according to its state of subdivision. Detach the flask, and remove the solvent by means of a stream of dry air with the necessary precautions to prevent detonation of nitroglycerin in the extract. Dissolve the residue in 60 ml. of glacial acetic acid, and add 25 ml. of 0·1 *N* bromate-bromide (2·784 g. of potassium bromate and 15 g. of potassium bromide per litre) from a pipette. Add 5 ml. of concentrated hydrochloric acid, stopper the flask, and mix the contents. Allow the bromination to proceed for 1 ± 0·25 min. from the time of acidification if centralite or diphenylamine is being determined, or at least 5 min. for acardite. Add 10 ml. of 15 per cent. potassium iodide solution, swirl the flask, and rinse down the gutter and walls of the flask with distilled water. Titrate the solution immediately with 0·05 *N* sodium thiosulphate, adding 5 ml. of 0·5 per cent. starch solution as the end-point is approached. Make a blank determination with 60 ml. of glacial acetic acid. The amount per cent. of stabiliser in the sample is given by the expression $\frac{(1 - A/B)NVC}{W}$, where A (ml.) is the volume of thiosulphate solution consumed in titration of the sample, B (ml.) is the volume of thiosulphate solution consumed in the blank determination, N is the normality of the bromate-bromide solution, V (ml.) is the volume of the pipette, W is the wt. of sample (g.) and C is one-tenth of the equivalent weight of stabiliser (6·709 for centralite, 2·115 for diphenylamine, and 5·306 for acardite).

Nitroglycerin remaining in the solution at the end of the titration should be destroyed by boiling with an excess of ferrous chloride, or by some other appropriate method. Nitroglycerin and diethyl phthalate do not interfere with the method. The method in the form given is not applicable in presence of more than one stabiliser. Typical data are recorded. A. O. J.

Inorganic

New Principle of Fractionation of the Rare Earths with Nitrilo-triacetate. Separation of Lanthanum and Cerium. G. Beck (*Helv. Chim. Acta.*, 1946, **29**, 357–360)—The formation of stable complexes by nitrilo-triacetic acid was reported by Pfeiffer and Offerman (*Ber.*, 1942, **75**, 1), and G. Schwarzenbach *et al.* (*Helv. Chim. Acta*, 1943, **26**, 418, 452; 1945, **28**, 828, 1133). Their extreme stability is exemplified by the fact that the alkaline earth derivatives give precipitates only slowly with oxalates and carbonates, and suggests the suitability of using this reagent for separating the rare earths. Their difficultly-soluble oxalates and fluorides dissolve readily in weakly alkaline solutions of nitrilo-triacetic acid, and are reprecipitated by cautious acidification. The lanthanum salt, probably $Na_3[La(N(CH_2COO)_3)_2]$, is the least stable of the series, and gradual addition of ammonium oxalate precipitates a lanthanum-containing oxalate at a *p*H of about 6. Praseodymium and neodymium oxalates come down on further acidification, and samarium at about *p*H 5, gadolinium at approximately *p*H 4·5, and erbium at *p*H 4. These *p*H values apply in the presence of a large excess of reagent, otherwise precipitation occurs at higher *p*H.

Quadrivalent cerium in weakly alkaline sodium nitrilo-triacetate gives with hydrogen peroxide an orange, hydrated ceric oxide. The decrease in *p*H due to the liberation of acid is compensated for by adding ammonia. The cerium is free from didymium, and the reaction is selective, and sensitive to 8 γ of cerium per ml. Under similar conditions lead salts give a yellowish-brown precipitate, which is soluble in an excess of reagent; titanium gives no colouration; manganese is not oxidised by peroxide; thallium gives a precipitate under certain conditions, but both the soluble and the insoluble complexes give thallous iodide with potassium iodide. At *p*H 9 to 10, the rare earth nitrilo-triacetates give hydroxides or basic salts, lanthanum precipitating first and in a much enriched condition. Cerium, like titanium (*p*H 4·5), thorium, and zirconium (<*p*H 5·4) is soluble in weakly acid solutions. At *p*H 5·5, the zirconium complex is sufficiently stable to hinder precipitation by phosphate ions; at *p*H 5·3, precipitation of phosphate commences and increases with decreasing *p*H. Thorium dissolves in acetic acid solutions of nitrilo-acetate, and oxalate ions give no precipitate until the solution is acidified with hydrochloric acid to *p*H 3·8. Zirconium gives no precipitate under these conditions.

Precipitation of lanthanum—From a nitric acid solution of lanthanum oxide, containing about 10 per cent. of praseodymium and neodymium, precipitate the impure oxalates completely with saturated ammonium oxalate solution, dissolve the precipitate in weakly alkaline sodium nitrilo-triacetate solution, and acidify to *p*H 6·6 with 30 per cent. acetic acid to precipitate lanthanum oxalate. The oxalate thus obtained gives on ignition a pure, white oxide,—about 80 per cent. of the impure material, which after dissolution in nitric acid, gives no praseodymium spectrum and only the strongest of the neodymium lines. The remaining 20 per cent. is thrown down at *p*H 4·3 as an oxalate that yields a brownish oxide on ignition.

A solution of technical didymium oxide, containing much praseodymium, neodymium, and samarium, in 40 ml. of sodium nitrilo-triacetate solution is treated with 30 ml. of ammonium oxalate solution, and brought to *p*H 5·9 by means of acetic acid. The oxalate obtained gives about 10 per cent. of pure white oxide containing no praseodymium or neodymium . Addition of another 30 ml. of oxalate solution to the filtrate (*p*H 5·5) gives a precipitate which yields 15 per cent. of blue-white oxide containing some neodymium. A fraction at *p*H 5 yields a blue oxide containing traces of praseodymium. The next fraction contains much praseodymium. Thus, neodymium is precipitated between lanthanum and praseodymium. Absorption lines are moved slightly towards the red end of the spectrum in the nitrilo-triacetate complex.

The method can be applied also to samarium-gadolinium mixtures. Gradual acidification of a solution of the mixture in the alkaline reagent results at *p*H 5·5 to 6·9 in a precipitate giving neodymium and samarium spectra; at *p*H 5·2 to 4·4, in a precipitate giving only a weak neodymium spectrum; and at *p*H 3·4 to 3·0, in one giving only very weak spectra. On combining the precipitates obtained at *p*H 4·4 to 3 and reacidifying to *p*H 3, about 4·5 per cent. of the original material, giving a colourless solution and very faint spectra, and consisting of pure gadolinium is obtained. Earths of the erbium series precipitate at a much lower *p*H than those of the cerium series.

A solution of sodium lanthanum nitrilo-triacetate gives with cobalt hexammino nitrate an insoluble precipitate of yellow rhombic crystals of the constitution $[Co(NH_3)_6][La\{N(CH_2.COO)_3\}_2]$. Other rare earths give similar precipitates, but there is a tendency to supersaturation. Cerium gives hexagonal plates, thallium forms in a micro-crystalline condition, but thorium, zirconium, aluminium, iron, and bismuth give no precipitates. M. E. D.

Anomalous Behaviour of some Oxidising Agents. R. K. McAlpine (*J. Chem. Educ.*, 1946, **23**, 301–305)—From a consideration of the oxidation potentials and corresponding half-reactions of sodium hypochlorite, hydrogen peroxide, and potassium persulphate, it is to be expected that the efficiency of potassium persulphate as an oxidising agent will be unaffected by the acidity of the medium, whilst the reduction of the other reagents is accompanied by the production of hydroxyl ions. Thus, at *p*H 9, the persulphate is much the strongest oxidising reagent, while hydrogen peroxide is slightly the stronger of the other two. The reducing substances used were the manganous ion, the cobaltous ammonium ion, and nickelous hydroxide. The concentrations of the oxidising solutions were sodium hypochlorite 1·3 *N*, hydrogen peroxide 1·7 *N*, and saturated potassium persulphate 0·3 *N*. Theoretically, all three reagents should oxidise the manganous ion through the tervalent state to manganese dioxide, the cobaltous ammonium ion to the cobaltic ammonium ion, and the nickelous hydroxide to nickelic oxide.

For the oxidation of manganese, 5 ml. of each oxidising solution were added to 25-ml. portions of a solution containing 10 mg. of $Mn^{\cdot\cdot}$, 5 ml. of 5 *N* ammonium chloride, and 10 ml. of 5 *N* aqueous ammonia. Peroxide gives instantly a dark-brown precipitate with considerable frothing; persulphate gives slowly a brown precipitate, which turns black. The hypochlorite gives a similar effect, but the precipitate redissolves on swirling to form a faintly purple solution; on long standing, a dark brown precipitate is produced. Precipitation is complete in all but the hypochlorite-treated solution. The anomalous behaviour with hypochlorite is discussed later.

The solution of nickel contained 50 mg. of bivalent nickel in 15 ml., and 5 ml. of 3 *N* sodium hydroxide were added to form a light green precipitate. On adding 5 ml. of the oxidising agents to 20-ml. portions, persulphate instantly gives black nickel dioxide, hypochlorite gives almost as rapid an oxidation, whilst peroxide gives no change until its catalytic decomposition is complete, leaving a precipitate slightly darker in colour than the original nickelous hydroxide. Precipitation of nickel hydroxide with 15 ml. of 3 *N* sodium hydroxide in the presence of 5 ml. of 5 *N* ammonium chloride, 10 ml. of 5 *N* aqueous ammonia solution, and 5 ml. of the oxidising solution gives with persulphate a black precipitate; with hypochlorite, green nickelous hydroxide; and with peroxide, a green coloration, but no precipitate. After about 15 min. precipitation is complete, however.

The experimental cobaltous ammonium ion solution contained 10 mg. of bivalent cobalt, 5 ml. of 5 *N* ammonium chloride solution, and 10 ml. of 5 *N* aqueous ammonia in a total volume of 25 ml. Peroxide gives a deep red coloration rapidly; hypochlorite gives slowly a lighter coloured solution; persulphate forms primarily a blue-green solution, which turns dark red, showing an indirect reaction.

The anomalous behaviour of hypochlorite with manganese and nickel in ammoniacal solution is due to the intermediate formation of hydrazine, a powerful reducing reagent, which results in the redissolution of manganese in the reduced state. The slight purple coloration is attributed to the presence of colloidal $Mn(OH)_3$, oxidation to permanganate being unlikely in the strongly alkaline solution.

The reducing power of hydrazine was tested on manganese dioxide suspension, nickelous hydroxide treated in the absence of ammonia with hypochlorite, and nickelic oxide in the presence of persulphate and ammonia. The cobaltic ammonium ion was found to give no immediate colour change, although the oxidation potential is between those of nickelic oxide and $Mn(OH)_3$, which are both easily reduced. Oxidation of the cobaltous ammonia ion in the presence of hydrazine shows no variation with peroxide, but persulphate gives more slowly a red colour without the intermediate blue-green colour. Hypochlorite gives a distinct effervescence with a slight deepening of colour. This last reaction proceeds according to the theory that the oxidation of hydrazine to nitrogen takes place more readily than that of the cobaltous ammonia ion.

In the qualitative separation of manganese, nickel, cobalt, and zinc, the first precipitation of manganese in ammoniacal solution is best effected by hypochlorite, since it carries down a negligible amount of cobalt. Also precipitation of nickel in the filtrate by an excess of sodium hydroxide then shows some manganese and a trace of cobalt; if the other reagents are used, considerable cobalt is carried down although the precipitate is free from manganese. The interference of manganese in testing for nickel can be avoided by using acetic acid solution, or by removing manganese dioxide before applying the test in ammoniacal solution. The poor separation of cobalt from manganese and nickel when using persulphate is due to co-precipitation of colloidal cobaltic hydroxide with nickel dioxide but not with the hydroxide. The formation of colloidal nickelous hydroxide when using alkaline peroxide assists in the decomposition of the excess peroxide, which occurs less readily in the absence of ammonia. M. E. D.

Microchemical

Micro-separation of Zinc and Aluminium by means of Potassium Ethyl Xanthogenate. P. Wenger, E. Abramson, and Z. Besso (*Helv. Chim. Acta,* 1946, **29**, 49–51)—The method depends on the quantitative precipitation by potassium ethyl xanthogenate, $KSCSOC_2H_5$, of zinc, aluminium being left in solution. The zinc xanthogenate is dissolved in acetic acid, and the zinc determined as the 8-hydroxyquinoline complex according to the method of Cimermann and Wenger (*Mikrochem.,* 1938, **24**, 148), or as the pyrophosphate.

Qualitative experiments showed that the conditions for obtaining the best results were as follows. A freshly prepared 2 per cent. solution of potassium ethyl xanthogenate must be used, untrustworthy results being obtained with a solution 24 hours old. Precipitation should be effected at *p*H 5 to 5·5, 1·2 ml. of reagent being used for each 2 mg. of zinc present, but not less than 0·9 ml. for smaller amounts. The reagent must be added dropwise with vigorous stirring, and the first-formed, voluminous precipitate left to stand until it is crystalline (10 to 25 min.), then filtered immediately, and washed with a 2 per cent. solution of the reagent.

Separation of zinc and aluminium. Method—To a solution containing 2·5 to 5 mg. of zinc and aluminium in an unweighed Emich beaker, add 1 drop of Merck's universal indicator, 2 drops of 10 per cent. acetic acid, and 40 per cent. aqueous sodium acetate solution until the solution is at *p*H 5 to 5·5. Add, drop by drop, 1·2 ml. of a 2 per cent. solution of the reagent for each 2 mg. of zinc present, and stir vigorously with a micro-rod; rinse the rod with 4 drops of the reagent. A flocculent precipitate forms rapidly, but on standing for 10 to 25 min. the turbid solution clears and the precipitate becomes compact and crystalline. Filter immediately into a weighed Emich beaker containing 0·4 ml. of 90 per cent. acetic acid to prevent hydrolysis of the aluminium acetate, and wash the precipitate three times with 6 drops of reagent. Concentrate the filtrate on the water-bath until the solution contains about 1 mg. per ml.; disregard a temporary turbidity during the evaporation. To the unweighed beaker, add 0·5 ml. of distilled water and 10 drops of 10 per cent. acetic acid to effect dissolution of the zinc. Filter into a tared beaker if the determination of zinc is to be made with 8-hydroxyquinoline, or into a tared crucible if the zinc is to be weighed as pyrophosphate. Wash the beaker and filter-stick with 1 ml. of distilled water.

Determination of zinc—(*a*) *8-hydroxyquinoline method*—Concentrate the zinc acetate solution to 1·5 ml. and then proceed according to Wenger and Cimermann (*loc. cit.*).

(*b*) *Pyrophosphate method*—Set the beaker containing the zinc acetate solution on the water-bath and add dropwise 1 ml. of 5 per cent. ammonium phosphate for each milligram of zinc present, 1 drop of universal indicator, and 2 to 3 drops of 1 per cent. aqueous ammonia, thus obtaining a *p*H of 6. Digest hot for 30 min., and cool for 30 min. before filtering and washing with 1 per cent. ammonium nitrate and 50 per cent. alcohol alternately. Dry in an oven, transfer to a muffle furnace, and raise the temperature slowly to 900° C., and heat for 5 min. Cool on a nickel block, and weigh after 30 min.

If the aluminium content is greater than 50 per cent. the pyrophosphate method is preferable, since the 8-hydroxyquinoline method tends to give slightly low results.

Determination of aluminium—The 8-hydroxyquinoline method may be conveniently employed since the aluminium is already in acetic acid solution, which should have a volume of several millilitres. Evaporate to about 1 ml. and acidify with 10 drops of 90 per cent. acetic acid, add 4 drops of 5 per cent. 8-hydroxyquinoline acetate solution for 1 to 3 mg. of aluminium, and heat on the water-bath. Buffer with 2 *N* ammonium acetate added dropwise until a permanent turbidity forms, and heat until the precipitate becomes crystalline; add a further 0·5 ml. of ammonium acetate and heat for 10 min. Filter hot, and wash 5 times with 0·3 to 0·5 ml. of boiling water before drying at 140° C. Weigh after 30 min.

Results—A hundred analyses with samples containing varying proportions of zinc and aluminium were completed, accurate results being obtained within the limits zinc, 25 to 75 per cent. and aluminium, 75 to 25 per cent. M. E. D.

Semimicro-Detection of Thiocyanate and Halide Ions. L. E. Porter (*J. Chem. Educ.*, 1946, **23**, 402)—In the detection of halides in the silver nitrate group, cyanogen anions and sulphide interfere. The ferro- and ferri-cyanide, and sulphide ions can be removed as their zinc salts (Abegg and Herz, *Z. anorg. Chem.*, 1900, **23**, 236; Weber and Winkelman, *J. Amer. Chem. Soc.*, 1916, **38**, 2000), or as their cadmium salts (Duschak and Sneed, *J. Chem. Educ.*, 1931, **8**, 1386; Pierce and Hazard, *Ibid.*, 1944, **21**, 126) before the silver nitrate is added. In the absence of cyanide, only thiocyanate and halides then remain to be precipitated. Available methods of separating thiocyanate and bromide tend to result in loss of bromide.

The following separation depends on the formation of a complex ferric thiocyanate, probably $FeSCN^{\cdot\cdot}$, when the precipitated silver thiocyanate is suspended in aqueous hydrochloric acid and treated with ferric chloride, the bromide remaining unchanged. The reaction proceeds readily, probably according to the scheme

$$AgSCN + Fe^{\cdot\cdot\cdot} + Cl' \longrightarrow FeSCN^{\cdot\cdot} + AgCl.$$

Since the separation and detection of silver thiocyanate are simultaneous, completeness of removal is easily recognised; and the subsequent test for bromide is more certain.

Procedure—Remove ferrocyanide, ferricyanide, and sulphide ions from the solution as their zinc or cadmium salts, acidify the solution with nitric acid, and add a slight excess of silver nitrate solution. Separate and wash the precipitate, and extract it with 1 ml. of 3 *M* ammonium carbonate. Test the extract for chloride by adding an excess of dilute nitric acid. The residue may contain thiocyanate, bromide, iodide, and some chloride of silver. Extract it with 7·5 *M* ammonia and detect iodide in the residue by reduction with zinc and dilute sulphuric acid, and addition of potassium nitrite and carbon disulphide to the filtered solution. Acidify the ammoniacal solution from the iodide separation with dilute nitric acid and filter. Wash the precipitate, and treat it with 1 ml. of water, 5 drops of 6 *M* hydrochloric acid and 5 drops of 2 *M* ferric chloride, and stir thoroughly. A red to reddish-brown solution indicates thiocyanate. Allow to stand for 3 to 4 min., and repeat the treatment with acid and ferric chloride until no further coloration is produced; one repetition is usually sufficient. Warm the residue with water containing a few drops of dilute nitric acid to remove the iron, and detect bromide by reducing it with zinc and dilute sulphuric acid, and adding hypochlorite and carbon disulphide. M. E. D.

Physical Methods, Apparatus, etc.

Chromatography in the Separation and Determination of the Basic Amino Acids. M. S. Bergdoll and D. M. Doty (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 600–603)—The individual basic amino acids can be separated chromatographically from amino acid mixtures and protein hydrolysates by means of Lloyd's reagent (fuller's earth). Hydrochloric acid was the most effective eluant for spreading the lysine, histidine, and arginine bands, and it removed lysine completely before removing any histidine or arginine; it was therefore used as the first eluant. Sodium bicarbonate solution was selected as the second eluant because it separated histidine from arginine. Pyridine solution was used to remove arginine.

Procedure—Adjust the acidity of the amino acid solution, 10 ml. of which contain 1 to 6 mg. of lysine, 1 to 4 mg. of histidine, and 1 to 8 mg. of arginine, to about 0·3 *N* with hydrochloric acid, add 30 to 50 mg. of zinc dust for each 10 ml. of solution, heat to 80° C. and cool. Pack an adsorption tube, 400 mm. long and 19 mm. in diameter, with 20 g. of a mixture (1:2) of Lloyd's reagent and Hyflo Super-Cel and put a 0·6-cm. layer of Hyflo-Super Cel on top of the adsorbent mixture. With the aid of suction, pass the following liquids in succession through the column: 50 ml. of 1·7 *N* hydrochloric acid, 10 ml. of the amino acid solution, 180 ml. of 0·5 *N* hydrochloric acid (using the first 10 ml. to rinse the amino acid solution on to the adsorbent), 200 ml. of *N* hydrochloric acid, 150 ml. of 0·125 *M* sodium bicarbonate, 100 ml. of 10 per cent. pyridine in 0·7 *N* hydrochloric acid, and 40 ml. of 0·5 *N* hydrochloric acid. Add each new solution just as the last of the previous solution is about to disappear into the adsorbent.

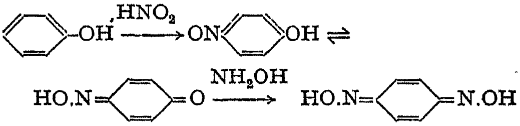
Change the receiver when a total of 80, 275, 480, 625, and 730 ml. of solution has been added to the column of adsorbent, and discard the first fraction. The second fraction contains the non-basic amino acids and ammonia, the third lysine, the fourth histidine, and the fifth arginine. Run a blank column in a similar manner, with 0·3 *N* hydrochloric acid treated with zinc replacing the amino acid solution. This serves as a reagent blank for the individual amino acid estimations.

Estimate the histidine by means of Pauly's diazo reaction (*cf.* Macpherson, *Biochem. J.*, 1942, **36**, 59), and the arginine by means of Sakaguchi's reaction (*cf.* Doty, *Ind. Eng. Chem., Anal. Ed.*, 1941, **13**, 169). The following method is recommended for the estimation of the lysine: make the solution just acid to phenolphthalein, and add 15 ml. of a phosphate buffer solution, *p*H 7·4 (50 ml. of 0·4 *M* potassium dihydrogen phosphate and 4·1 ml. of 1·0 *N* sodium hydroxide diluted to 100 ml.). Transfer a measured volume, containing 0·02 to 0·10 mg. of lysine, to a 25-ml. flask and make up to 3 ml. with the blank solution. Add 1 ml. of ninhydrin solution (5 mg. of triketohydrindene hydrate, 0·4 mg. of sodium hydroxide and 263 mg. of sodium chloride per ml.), and 5 ml. of glycerol. Mix, immerse for 30 min. in boiling water, cool, and dilute to a suitable volume with 95 per cent. ethanol. Evaluate the colour in a photo-electric colorimeter, and calculate the lysine concentration from a standard curve.

The recoveries of the basic amino acids when adsorbed from amino acid mixtures were 103 ± 2 per cent. for lysine, 100 ± 1 per cent. for histidine, and 99 ± 2 per cent. for arginine. The lysine and histidine values found for proteins were generally higher than the values recorded in the literature, although they were in closer agreement with the more recent results. The arginine values compared favourably with those given in the literature.

F. A. R.

Chromatographic Resolution of the Quinone Oximes. D. K. Gullstrom, H. P. Burchfield, and J. N. Judy (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 613–616)—*p*-Benzoquinone dioxime is prepared by nitrosation of phenol and treatment of the resulting monoxime with hydroxylamine:



The monoxime can be separated from the dioxime and from the by-products of the reaction by chromatography on alumina.

Procedure—Dissolve a 1-g. sample of the reaction mixture in 100 ml. of acetone by heating under reflux, filter, and run the filtrate, after concentration if necessary, through a column, 15 cm. × 18 mm., of a mixture (9:1) of activated alumina and Hyflo Super-Cel. Wash with 10 ml. of 1 per cent. v/v acetic acid in acetone and then with 300 ml. of 5 per cent. v/v methanol in acetone. Remove the top brown layer by means of a spatula and discard. Transfer the green zone, which contains the monoxime, to a 250-ml. beaker and elute with successive portions of *N* sodium hydroxide, using a total volume of 100 ml. Filter, dilute the filtrate to 1 litre with water, and then dilute again to a volume suitable for measuring the optical density at 399 and 363 mμ. The green zone contains all the monoxime together with 20 to 35 per cent. of the dioxime, for which a spectrophotometric correction is applied. The percentage of monoxime is given by the expression:

$$\frac{100\, V(K'\lambda_2.D\lambda_1 - K'\lambda_1.D\lambda_2)}{G.F.(K'\lambda_2.K\lambda_1 - K'\lambda_1.K\lambda_2)}$$

where $D\lambda_1$ and $D\lambda_2$ are the measured optical densities at 399 and 363 mμ, respectively, *G* is the weight of the sample, *V* is the volume (litres) to which the solution is diluted, $K\lambda_1$ and $K\lambda_2$ are the specific extinction coefficients of the monoxime at 399 and 363 mμ, respectively (224 and 109, respectively), $K'\lambda_1$ and $K'\lambda_2$ are the specific extinction coefficients of the dioxime at 399 and 363 mμ, respectively (283 and 65·7, respectively), and *F* is the fraction of the monoxime recoverable after adsorption on alumina (generally about 0·95). When a known amount of the monoxime was added to a reaction mixture, the average deviation from the theoretical result was ± 0·23 per cent. The precision of the method, as judged by the results of duplicate analysis of 64 samples containing 2 to 55 per cent. of monoxime, was ± 3·6 per cent. of the amount present. F. A. R.

Conditions Affecting the Sequence of Organic Compounds in Tswett Adsorption Columns. H. H. Strain (*Ind. Eng. Chem., Anal. Ed.*, 1946, 18, 605–609)—It has become the practice to identify and name the components of a mixture by their relative positions on Tswett adsorption columns, and predictions concerning the structure of an organic compound have sometimes been made from its adsorptive power relative to those of known substances. It has now been demonstrated that the relative adsorption sequence on such columns varies with circumstances. Most of the work was carried out with binary mixtures, but mixtures containing 3 or more components have also been examined. Several factors affect the sequence of adsorption, and in the original paper many examples are given of the reversal of two bands. These factors are: the nature of the solvent; the presence of an impurity in the solvent; the hydrogen ion concentration; the concentration of the solutes; the nature of the adsorbent; and the temperature. This variation of the adsorption sequence with changes of conditions in the adsorption columns can often be used to advantage. Thus, the identity or non-identity of two substances may be established with greater certainty by adsorbing a mixture of the two on different adsorbents and elution with different eluants than by adsorption on one column only. Secondly, when attempting to purify a substance chromatographically, it is an advantage that the band corresponding to it should be below that corresponding to the impurity; a substance forming an upper band should therefore be readsorbed under conditions such that it forms the least readily adsorbed band.

Clearly, the relationship between adsorbability and chemical structure is more complex than was formerly supposed, and adsorbability apparently depends on competition of solvent, solution, and adsorbent for one another. Since recent work has shown that the rate of migration of a solute through an adsorption column is a function of the adsorption isotherm, variations of the adsorption sequence with different conditions in the columns must result from disproportionate variation of the adsorption isotherms. F. A. R.

Use of High-frequency Oscillators in Titrations and Analyses. F. W. Jensen and A. L. Parrack (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 595–599)—When a high-frequency oscillator is loaded by the introduction of a liquid or a solution into its tank circuit, its characteristics are altered. The magnitude of the change in the oscillator current is affected by the volume of the solution, the location of the solution within the circuit, and the conductivity of the solution due to its ionic or dipole concentration. The changes in conductivity during a reaction cause variations in loading whereby the course of the reaction can be followed, and an oscillator that is suitable for applying this principle to analytical work is now described. By the use of this instrument, the end-point in several types of titration can be determined.

A tuned-plate, tuned-grid oscillator with a sensitive metering system in the positive power supply lead is used, and details of the circuit are given. Frequencies in the range 15 to 20 megacycles give satisfactory results. The tube containing the liquid is surrounded by a shield extending well below the liquid level and is placed inside the oscillator coil. The progress of the reaction is followed by measurement of the oscillator current, and for a titration, a change in slope of the curve obtained by plotting current against the volume of titrant marks the end-point.

Results are given for a series of titrations in which 25 ml. of a 0·1 *N* solution were diluted with 100 ml. of water and titrated with another solution approximately 0·1 *N*. In titrating solutions of hydrochloric acid with sodium hydroxide or sodium carbonate; of phosphoric acid with sodium hydroxide; and of potassium chloride with silver nitrate, the current-titre curve is V-shaped, the sharp point of inflexion being the end-point of the titration. The curve for the titration of sodium carbonate solution by 0·1 *N* hydrochloric acid gave two points of inflexion, and in the titration of acidified ferrous ammonium sulphate solution with potassium permanganate solution, addition of the titrating agent beyond the end-point lowered the conductivity of the solution and so the current continued to fall beyond the point of inflexion. The V-shaped graph was obtained if the potassium permanganate used was made 0·4 *N* with respect to potassium sulphate. Potassium permanganate-sodium oxalate titrations showed similar characteristics. Titration of *o*-phthalic acid in acetone with sodium methylate in methanol gave a sharp end-point.

The end-points obtained were compared with the known end-points for the various titrations and good agreement was found; *e.g.*, silver nitrate - potassium chloride, known 24·92 ml., observed 24·90 ml.; hydrochloric acid - sodium carbonate, known 12·20, observed 12·18 and 24·40 ml. For two types of acid-alkali titrations, the end-points were compared with those obtained by the usual conductance method; agreement was again satisfactory. With solutions in organic solvents, loading due to rotation of dipoles is an appreciable part of the total effect, and an end-point can be obtained only by suitable choice of solvent.

Because silver chloride becomes more soluble as the equivalence point is approached, and dilution of ions takes place as titration proceeds, the titration of 25 ml. of 0·01 *N* potassium chloride, diluted with 300 ml. of water, by 0·01 *N* silver nitrate gave a minimum conductivity point at 23·90 ml. instead of at 25·00 ml., the true end-point. By using conductivity and solubility data, the amount of silver still in solution at the minimum point can be calculated, and the corrected end-point thus obtained was 24·99 ml.

As the curve obtained by plotting oscillator current against the concentration of hydrogen chloride in solution in dry benzene is linear, and a change in concentration from 0 to 0·275 per cent. by weight of hydrogen chloride corresponds to a change of 100 microamp., a sensitive method for determining hydrogen chloride in benzene becomes available. B. A.

Spectrophotometric Changes during Oxidation of Vitamin A Oils. G. R. Halpern (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 621–625)—Fish liver oils containing vitamin A are essentially solutions of the esters of that vitamin in a fatty carrier oil. Knowledge of the quality of the carrier oil is important for the evaluation of vitamin A oil, since carrier oils containing degradation products due to oxidation have considerable influence on the extinction coefficient at 328 mμ and on the whole vitamin A curve. The vitamin A ester itself has a sharp maximum at 328 mμ. Biologically inactive substances that may accompany it do not show this sharp peak, but have maxima at wavelengths sufficiently near to 328 mμ to produce considerable absorption at this point. Oxidised oils can also inhibit the action of vitamin A *in vivo*.

The method of Oser *et al.* (*Ibid.*, 1943, **15**, 717) of plotting absorption curves of vitamin A oils in terms of absorption ratios could be useful in detecting anomalous or oxidised oils, but the method for measuring the ratio $E^{1\%}_{1cm.}$ 300/328 mμ with the limit of 0·72 for acceptable oils did not prove satisfactory, since it was found that an oil could be considerably oxidised and still give the accepted ratio. Oser *et al.* (*Ibid.*, 1945, **17**, 559) showed that oils with an acceptable $E^{1\%}_{1cm.}$ 300/328 mμ ratio did not always show the biological potency corresponding to their $E^{1\%}_{1cm.}$ 328 mμ values. It seemed desirable, therefore, to study spectrophotometric curves of the saponifiable and unsaponifiable components of the carrier oil, apart from vitamin A, during accelerated oxidation to establish their influence upon the absorption curve of vitamin A oils, and to develop better criteria for the evaluation of these oils.

Freshly prepared samples of gray fish (*Squalus suckleyi*) liver oil and ling cod (*Ophiodin elongatus*) liver oil were oxidised to different degrees by immersing them for varying periods in a water-bath at 75° C., and the absorption curves were determined in the region from 220 to 400 mμ for the whole oil and for the unsaponifiable fraction, the difference representing the absorption of the saponifiable carrier. The absorption of that part of the oxidised vitamin A which is washed out during the saponification (Oser *et al.*, *loc. cit.*, 1943) is also included in the subtracted curve. To obtain the curve of the unsaponifiable carrier, the unsaponifiable fraction was destructively irradiated by exposure in a water-cooled cell at 20 cm. for 1 hr. to a Uviarc amp (UA 32A6) with a No. 597 Corning filter. On the assumption that only vitamin A is destroyed by irradiation the curve obtained after irradiation shows the absorption of the unsaponifiable carrier, and, by subtracting the absorption values of the irradiated unsaponifiable fraction from those of the original unsaponifiable fraction, the vitamin A curve can be obtained. Values so obtained are referred to as the modified values (Little, *Ibid.*, 1944, **16**, 288). The spectrophotometric analyses were made with a Beckman quartz spectrophotometer with an ultraviolet phototube and, as light sources, a hydrogen discharge tube below 320 mμ and a tungsten lamp above 320 mμ, and all determinations were made in quartz cells with purified *iso*propanol as solvent. The saponifications were made essentially by the procedure of Oser *et al.* (*loc. cit.*, 1943), blank saponifications made on the reagents being used as spectrophotometric blanks.

With the saponifiable carrier, new maxima are formed during oxidation at 235, 275 to 280, and 335 to 345 m μ, and the shape of the curve resembles that of the curves of rancid and oxidised fats. The appearance of new maxima during oxidation is generally attributed to formation of different conjugated systems, produced by isomerisation of unconjugated fats and by destruction of a part of the four conjugated double bonds of vitamin A. Both components of the oxidised saponifiable carrier (fatty acid and oxidised vitamin A) can therefore be responsible for the formation of the 275 mμ band, the extinction coefficients of conjugated fatty acids being of a high order. The vitamin A curve is affected by formation of these three maxima. The minimum at 260 mμ steadily increases during oxidation, despite loss of potency, and this causes broadening of the curve, increase in the $E^{1\%}_{1cm.}$ 300/328 ratio, and a shifting of the 328 mμ peak to a lower wavelength in the later stages of oxidation. Examination of the curves of the saponifiable carrier shows that the point at 260 mμ (the minimum of the vitamin A curve), or at about 280 mμ (the maximum of the saponifiable carrier curve) is a better location for taking the ratio than the 300 mμ point. At these points the $E^{1\%}_{1cm.}$ values increase during oxidation and, at the same time, those at 328 mμ decrease, whereas at 300 mμ the values remain almost constant. (The point at 300 mμ was apparently chosen empirically to avoid use of a hydrogen discharge lamp.) When the ratios $E^{1\%}_{1cm.}$ 300/328, 280/328, and 260/328 mμ of the oxidised oils are plotted against loss of vitamin A, the curves for the 260 and 280 ratios are steeper.

The extraneous absorption (per cent. of the absorption of the whole oil) of the saponifiable carrier steadily increases during oxidation, and could be used as a criterion for the quality of vitamin A oils. At 260 and 280 mμ the extraneous absorption increases significantly during the first stages of oxidation. It is most pronounced at 260 mμ because the influence of the 325 mμ maximum is greater than at 280 mμ, but both points may be used as criteria, and the 260 mμ point has the advantage that the determination can be made more accurately on the flat portion of the curve.

The determination of vitamin A on the unsaponifiable fraction of the carrier is sufficient where the extraneous absorption of the saponifiable carrier does not change considerably during the first stages of oxidation, and commercially-prepared oils, which are frequently slightly oxidised, would not give erroneously high values at 325 mμ when these are determined on the unsaponifiable fraction and calculated with a uniform factor. The vitamin A was destroyed in all samples by irradiation of the unsaponifiable fraction with the exception of a portion of ling cod liver oil which had been irradiated for 18 hr., and with both test oils a new maximum appeared at 270 mμ representing a substance formed from vitamin A during destructive irradiation (*cf.* Sobotka, *et al.*, *J. Amer. Chem. Soc.*, 1944, **66**, 1162; ANALYST, 1945, **70**, 28). The additional absorption of this substance affects considerably the curves of the modified values obtained by subtraction. During the oxidation of the test oils the absorption of the irradiated samples at 325 mμ showed a tendency to decrease, but the differences were within experimental error. It can therefore be assumed that no significant changes occur in the unsaponifiable carrier during the first stages of oxidation of either test oil, and the determination of the modified values is not justified.

The unsaponifiable carrier of the ling cod liver oil showed no pronounced maxima caused by components originally present in this fraction. The unsaponifiable carrier of grayfish oil, on the other hand, contains substances absorbing at about 238 mμ, partly masking the absorption of irradiated vitamin A at 270 mμ. The absorption at 238 mμ, however, does not affect the 325 mμ point, and remains constant in the first stages of oxidation, decreasing later. The main substance responsible for the absorption at 238 mμ was shown to belong to the glyceryl ether fraction, probably a higher unsaturated homologue of selachyl alcohol. Another substance of unknown constitution absorbing at this wavelength is present in very small amount.

Although the unsaponifiable carrier of the two test oils did not show oxidisable substances that would affect considerably the absorption curve of vitamin A, some fish liver oils can contain such substances included in the unsaponifiable fraction which produce an anomalous vitamin A curve.

In presence of such substances as kitol, vitamin A_2, anhydro-vitamin A and others, an accurate estimation of vitamin A by the means described is impossible. The commercial factor 2000 allows a certain amount of extraneous absorption in all oils because the crystalline vitamin A alcohol was shown to have a conversion factor of 2460 (Baxter and Robeson, *J. Amer. Chem. Soc.*, 1942, **64**, 2411). If the substances causing excessive extraneous absorption in the unsaponifiable fraction do not interfere with the Carr-Price reaction, colorimetric estimation of vitamin A can be used advantageously (Oser *et al.*, *loc. cit.*, 1945).

Considering the great difference in the ratios of both original test oils, it seems that the establishment of a definite limit for the magnitude of the ratio for the evaluation of the quality of fish liver oils is not satisfactory. A rigorous limit for good oils and a range for doubtful oils would be preferable. It is suggested that with doubtful samples the extraneous absorption per cent. of the saponifiable carrier, *viz.*,

$$\frac{E^{1\%}_{1\text{cm.}}\text{ (whole oil)} - E^{1\%}_{1\text{cm.}}\text{ (unsaponifiable fraction)}}{E^{1\%}_{1\text{cm.}}\text{ (whole oil)}} \times 100$$

at 280 or 260 $m\mu$ might be helpful in the estimation of the quality of these oils. From the present work, and from other experience, it appears that the value 0·72 for the $E^{1\%}_{1\text{cm.}}$ 300/328 $m\mu$ ratio indicates that oxidation has taken place especially with high potency oils. Further work, particularly biological, will be necessary to establish proper limitations of the values to be used as criteria. A. O. J.

Radioactive Studies. Utilisation of the Radioactive Isotope Dilution Procedures for Special Types of Chemical Problems. Determination of the Three Individual Components of Mixtures of Dibenzyl-Sulphide, -Sulphoxide, and -Sulphone as an Illustrative Example. F. C. Henriques, jun., and C. Margnetti (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 476–478)—As recent studies (Henriques, jun., *et al.*, *Ibid.*, 1946, **18**, 349, 415, 417, 420) have shown that some of the radioactive tracers most difficult to analyse can be determined to within 2 per cent., the accuracies of the isotope dilution method with either stable or radioactive isotopes are similar. This paper indicates the scope of the radioactive dilution method as applied to chemical problems, and gives an example of its use.

The two general procedures in applying the method are: (1) if the amount of non-radioactive compound, *P*, in the mixture is unknown, a known amount of the same compound containing a minute amount of tracer element, *P**, is added and dispersed uniformly. The amount of *P* present can be determined provided that a weighable amount of *P.P** can be isolated in a pure state; (2) if the amount of *P** in a mixture containing other radioactive compounds is unknown, then a weighed amount of *P*, sufficient to enable the isolation of a weighable quantity of pure *P.P**, is added, and the analysis becomes possible. Procedure (1) cannot be applied to the determination of traces, but (2) can be used to determine 10^{-3} μg. of some compounds.

The application of (1) to the analysis of a mixture of dibenzyl-sulphide, -sulphoxide, and -sulphone is described in detail. Samples of these compounds containing radioactive sulphur, ^{35}S, were prepared, and their radioactivities determined by the method described in an earlier paper (*Idem*, *ibid.*, 1946, **18**, 349). A weighed portion of each was added to the unknown mixture. Pure samples of each compound were isolated from the mixture by fractional crystallisation, and subjected to radioactive analysis. From the data obtained, the amounts present in the mixture were calculated. Analysis of a series of standard mixtures gave results to within ± 2 per cent. of the correct values. A paper to be published will describe the application of method (2) to the investigation of the mechanism of mustard gas vesication. B. A.

Radioactive Studies. Analytical Method for Determination of the Long-Life Carbon ^{14}C. F. C. Henriques, jun., and C. Margnetti (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 417–419)—Details are given of a procedure for measuring the radioactivity due to ^{14}C, half-life 1000 years, incorporated in organic compounds. The carbon-containing compounds are oxidised by a standard combustion procedure, and the carbon dioxide formed is introduced into an ionisation chamber attached to a Lauritzen electroscope. As little as 3×10^{-5} micro-curie of ^{14}C, diluted with 20 mg.-mol. of ordinary carbon, can be determined to within ± 2 per cent. The method is applicable to biological work in which ^{14}C is used as a tracer. B. A.

Radioactive Studies. Analytical Procedure for Measurement of the Long-lived Radioactive Sulphur ^{35}S, with a Lauritzen Electroscope, and Comparison of Electroscope with Special Geiger Counter. F. C. Henriques, jun., G. B. Kistiakowsky, C. Margnetti, and W. G. Schneider (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 349–353)—The paper describes a method for the determination of the radioactive tracer, ^{35}S, half-life 87 days. Samples of the sulphur-containing compounds are oxidised by the Carius method, and the sulphur is precipitated as benzidine sulphate. The radioactivity of the precipitate is measured by either a Geiger counter or a modified Lauritzen quartz-fibre electroscope. If the specific activity of the tracer is 1 milli-curie of ^{35}S per 10 mg. of compound, 10^{-3} μg. of any sulphur compound can be determined to within 2 per cent. Details of apparatus and procedure are given, and the utilities of the counter and electroscope compared. B. A.

Radioactive Studies. Analytical Procedure for Measurement of Radioactive Arsenic of 90-Day Half-life. F. C. Henriques, jun., and C. Margnetti (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 415–417)—The radioactive arsenic of 90-day half-life, believed to be ^{72}As or ^{73}As, is determined by hypophosphite reduction to the metal of the arsenic compound that is used as a carrier for this tracer element, collection of the precipitated arsenic on a filter plate, and measurement of its radioactivity by means of a Lauritzen electroscope. Details of apparatus and procedure are given. B. A.

Application of the Differential Manometer to the Measurement of Fluid Density and Specific Gravity with Special Reference to Temperature Effects. R. D. Cowherd (*Paper Trade J.*, 1946, **123**, Oct. 3, *T.A.P.P.I. Sect.*, 159–166)—In the instrument described, the density of a liquid is measured in terms of the difference in back-pressure set up when air from a common source is bubbled simultaneously through tubes immersed in a cylinder containing the liquid being tested and in a liquid of known density (*e.g.*, mercury), under standardised conditions (*e.g.*, of head). A standard type of flow-meter unit is used for the actual measurement of the pressure difference. Automatic temperature compensation is ensured by adjusting the weight of the comparison liquid and the diameter of the container so that any decrease in the density of the contents (due to a temperature rise) will exactly offset the increase in head due to the expansion which also results; and *vice versa*. This method is preferable to the insertion of the comparison liquid cylinder in the sample liquid cylinder, so as to form a concentric heat-exchanger. The subject is treated mathematically. J. G.

Isostatic Method for Determining the Gas Permeability of Sheet Materials. D. W. Davis (*Paper Trade J.*, 1946, **123**, Aug. 29, *T.A.P.P.I.*, *Sect.*, 97–104)—In the apparatus described, the gas to be tested is passed over one side of the test sheets which are held free from any face support in a diffusion cell, and a different "sweep" gas is passed over the other side; both gases are at approximately atmospheric pressure and at the same relative humidity. The sweep gas entrains any test gas that may permeate the test sheet, and the permeated gas is determined by chemical methods. For carbon dioxide permeability, the oxygen used as the sweep gas is passed through Ascarite, and the permeated carbon dioxide so absorbed is determined gravimetrically. For other test gases (*e.g.*, oxygen or nitrogen), the carbon dioxide used as the sweep gas is absorbed in potassium hydroxide solution, and the residual permeated test gas is determined by gas-volumetric methods. Data are given for a wide variety of sheet materials at various temperatures and relative humidities. J. G.

Reviews

CHEMICAL COMPOSITION OF FOODS. By R. A. MCCANCE and E. M. WIDDOWSON. Second Edition. Medical Research Council, Special Report Series No. 235. Pp. 156. London: H.M. Stationery Office. 1946. Price 6s.

Twice already, once on the first appearance of these tables and then when a third impression was produced, in 1940 and 1942 respectively, it has been my privilege and pleasure to welcome them in this journal on behalf of analytical chemists. Now I can do little more than repeat the substance of what was said on both those occasions.

The tables are as indispensable and authoritative as they are critically constructed by the authors on the basis of new analytical work scrupulously carried out by them. The new edition departs not at all in layout and arrangement of contents from the old, though it has additional information on a small number of foods. Thus, the data for bread, under Cereals and Cereal Foods, now include figures for "National Wheatmeal"—a defunct term already surrounded by an historical aura—as well as for white, this time specified as of 70 to 72 per cent. extraction, and for "whole meal," a term now properly used in quotation marks and defined as of 92 per cent. extraction; the reference to toasted Hovis has been omitted, presumably in part to avoid having to re-number everything subsequent. This re-numbering problem, and the preoccupation of the distinguished authors with many other lines of investigation, have doubtless prevented many additions to the original tables, though the insertion of fresh items, where space permits, could be indicated by small letters following the numbers, as already used in several places. Some space could, if it were essential, possibly be saved by removing a few of the more esoteric items; thus, to give figures for winkles purchased cooked and for winkles in shells as purchased is perhaps superfluous, the information being but rarely of great practical interest to the analyst or the dietician.

Perhaps the most interesting of the changes is the ruthless removal from this new edition of the table headed " 'Available' (Ionisable) Iron in Foods" and also of the reference to "availability" in the table giving figures for the phytic acid content of foods. Phytic acid phosphorus in the new table is expressed as a percentage of the total phosphorus: previously, figures were given for what was described with customary caution as "available" phosphorus —the quotation marks are the authors'—expressed in terms of the total phosphorus. One of the signs of increasing knowledge in physiological matters is frequently increased uncertainty —not necessarily an application of a certain much-discussed principle of modern physics.

There is one small criticism that a sympathetic reviewer would offer to the authors. When they produce further editions—and I would again express the hope that they may often be called upon to do so—they should, in their preface to those editions, call attention to any alterations to tabulated values that they have found it necessary to make, giving in the more important instances their reasons for the changes, where these are not obvious from inspection of the tables. This is, however, only a suggestion for an addition; I do not see how the would-be most carping of critics, with the utmost exercise of ingenuity, could suggest a single useful omission from this most valuable and valued compilation. A. L. BACHARACH

ADVICE TO AUTHORS

The Council has approved the following notice by the Publication Committee, which is here given in condensed form.

The Society publishes papers concerned with all aspects of analytical chemistry, inorganic and organic, including chemical, physical, biological, bacteriological and micro methods. Papers on these and allied subjects, by members of the Society or non-members, may be submitted for presentation and publication; they may:

(1) record proposals for new methods and the investigations on which the proposals are based;

(2) record the results of original investigations into known methods or improvements therein;

(3) record analytical results obtained by known methods where these results, by virtue of special circumstances, such as their range or the novelty of the materials examined, make a useful addition to analytical information;

(4) record the application of new apparatus and new devices in analytical technique and the interpretation of results.

Communications.—Papers (which should be sent to the Editor) will normally be submitted to at least one referee, on whose advice the Publication Committee will be guided as to the acceptance or rejection of any communication. Papers or Notes accepted by the Publication Committee may not be published elsewhere except by permission of the Committee.

Abstracts.—The MS. should be accompanied by a brief abstract of about 100 to 150 words indicating the scope and results of the investigation.

Proofs.—Proofs should be carefully checked and returned within 48 hours. Two galley proofs* will normally be sent out, one of which should be retained by the Author.

Reprints.—Ten Reprints are supplied gratis to the Author. Additional reprints may be obtained at cost if the Author orders them directly from the printers, W. Heffer & Sons Ltd., 104, Hills Road, Cambridge, at the time of publication. Details are sent to Authors with the proofs.

Notes on the writing of papers for THE ANALYST

Manuscript.—Papers and Notes should preferably be typewritten.

The title should be descriptive and should set out clearly the scope of the paper. Degrees are now omitted after the names of Authors in the headings of papers.

Conciseness of expression should be aimed at; clarity is facilitated by the adoption of a logical order of presentation, with the insertion of suitable paragraph or sectional headings.

Generally, the best order of presentation is as indicated below:

(*a*) General, including historical, introduction.

(*b*) Statement of object of investigation.

(*c*) Description of methods used. Working details of methods are usually most concisely and clearly given in the imperative mood, and should be given in this form, at least while economy of paper is pressing, *e.g.*, "Dissolve 1 g. in 10 ml. of water and add . . ." Well-known procedures must not be described in detail.

(*d*) Presentation of results.

(*e*) Discussion of results.

(*f*) Conclusions.

followed by a short summary (100 to 250 words) of the whole paper: items (*e*) and (*f*) can often be combined.

Illustrations, diagrams, etc.—The cost of setting up tabular matter is high and columns should therefore be as few as possible. Column headings should be brief or replaced by a number or letter to be used in combination with an explanatory footnote to the table.

Sketches or diagrams should be on white Bristol board, not larger than foolscap size, in Indian ink. Lettering should be in light pencil.

Tables or graphs may be used, but normally not both for the same set of results. Graphs should have the co-ordinate lines clearly drawn in black ink.

References.—References should be numbered serially in the text and collected in that order under "REFERENCES" at the end of the paper. They should be given in the following form:

1. Dunn, J. T., and Bloxam, H. C. L., *J. Soc. Chem. Ind.*, **1933**, **52**, 189T.
2. Allen, A. H., "*Commercial Organic Analysis*," Churchill, London, 1882.

Notes on the Presentation of Papers before Meetings of the Society are appended to the "ADVICE," copies of which may be obtained on application to the Secretary, 7/8, Idol Lane, London, E.C.3

*** During the paper shortage two copies of the MS. will not be insisted on, nor will two galley proofs be sent.**

MARCH, 1947 Vol. 72, No. 852

THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

An Ordinary Meeting of the Society was held at 6 p.m. on Wednesday, February 5th, at the Chemical Society's Rooms, Burlington House, London, W.1, with the President, Dr. G. W. Monier-Williams, in the chair. The following papers were presented and discussed: "Reductometric Determination of the Sulphoxide and Amine Oxide Groups," by Mrs. Erica Glynn; "The Determination of the Composition and Constitution of Ammonium Phosphomolybdate and the Conditions affecting its Precipitation," by W. P. Thistlethwaite, B.Sc., A.R.I.C.

NEW MEMBERS

Alan Bruce Anderson, B.Sc. (Adelaide), Ph.D. (Cantab.), M.R.C.S., L.R.C.P., F.R.I.C.; Frederick George Angell, B.Sc., Ph.D. (Birm.), F.R.I.C.; Fred William Chambers, Dip.Chem. (Cologne), A.R.I.C., A.M.I.Chem.E.; Ronald Powell Graham, M.A. (Ontario), A.M., Ph.D. (N.Y.); Alexander Donovan Kenny, B.Sc. (Lond.), A.R.C.S., A.R.I.C.; Miss Muriel Meiklejohn, B.Sc., A.R.I.C.; Oswald Neave, A.R.I.C.; Neville Newsam; Arthur Stanley Nickelson, B.Sc. (Lond.), A.R.I.C.; Anthony Rhys Phillips, B.Sc., A.R.I.C.; Leonard George Sherrington, M.Sc. (Lond.), A.R.C.S.; George Arthur Colvin Sirimanne, B.Sc. (Lond.), A.R.I.C.; Frederick Lloyd Warren, M.A., B.Sc.(Oxon.), Ph.D. (Lond.).

NORTH OF ENGLAND SECTION

A Meeting of the Section was held at Manchester on Saturday, October 19th, 1946. Mr. S. E. Melling presided over an attendance of thirty. The following papers were presented and discussed: "The Determination of Traces of Lead, Zinc and Tin in Phenol," by W. Hutchinson, A.R.I.C., and H. N. Wilson, F.R.I.C.; "Soil Biochemistry," by Dr. H. Lees, B.Sc., A.R.I.C.

The twenty-second Annual General Meeting of the Section was held in Manchester on Saturday, January 25th, 1947. The Vice-Chairman, Mr. C. H. Manley, presided over an attendance of thirty-five. The Hon. Secretary presented the Report and Financial Statement, which were adopted. Appointments of Officers and Committee members for the forthcoming year were made as follows:

Chairman, C. H. Manley. *Vice-Chairman,* J. G. Sherratt. *Hon. Secretary and Treasurer,* Arnold Lees. *Committee,* R. Crosbie-Oates, A. N. Leather, R. Mallinder, Norman Strafford, G. H. Walker and H. Weatherall. *Hon. Auditors,* U. A. Coates and J. R. Walmsley.

The following paper was read and discussed: "The Chemist in the Colonies," by J. F. Clark, M.Sc., F.R.I.C.

BIOLOGICAL METHODS GROUP

The Annual Meeting of the Biological Methods Group was held in the Rooms of the Chemical Society, Burlington House, Piccadilly, W.1, at 6 p.m., on Monday, 16th December, 1946. Mr. A. L. Bacharach presided and was re-elected as Chairman. The Vice-Chairman, Hon. Sec., Hon. Auditors, and Members of the Committee also remain as last year (Analyst, 1946, **71**, 201). The Committee's Report and the Financial Statement for the period 20th February—30th November, 1946, were presented and approved. The new Rules of the Group as approved by the Council were available at the meeting in proof form.

An Ordinary Meeting of the Group followed at 6.30 p.m., at which the following papers were read and discussed: "The Assay of Anti-Thyroid Substances, using Tadpoles," by Miss H. M. Bruce (whose remarks will be embodied in a paper to appear shortly in the Proceedings of the Royal Society) and "The Computation of Microbiological Assays of Amino-Acids and other Growth Factors," by Eric C. Wood (see page 84).

Linlithgow Library.

The Computation of Microbiological Assays of Amino-Acids and other Growth Factors

BY ERIC C. WOOD

(Presented at the Annual General Meeting of the Biological Methods Group on December 16th, 1946)

IT is the exception rather than the rule in microbiological assays that the mean response is linearly related to the dose. Nearly all workers find this relationship to hold in the two assays most frequently required—those for riboflavine and nicotinic acid by the Snell and Strong[1] and the Snell and Wright[2] techniques respectively, or such later modifications as those described in the recent Report of this Society's Sub-Committee on Vitamin Estimations.[3] Pyridoxine assays by means of *Neurospora* may also be of this type. But assays of amino-acids and of members of the vitamin B_2 complex other than those just mentioned, usually give smooth curves, with no linear portion, when the response is plotted against the dose (for typical examples, see Barton-Wright[4]). The result of plotting the response against the logarithm of the dose, instead of against the dose itself, is usually a curve also—a disappointing result in view of the large number of biological assays in which response is linearly related to logarithm of dose. Price[5] has found this latter relationship to hold in some of his riboflavine assays and another instance is mentioned below, but in general this relationship is not found in microbiological work.

A transformation that would give linear graphs would have the advantages that the accurate computation of the best estimate of the result is made easier, and a quick visual test of validity is afforded, besides which the further calculation of the standard error and of the confidence limits of the assay, when necessary, is much simplified.

While the literature of microbiological assay is rapidly becoming voluminous, there are regrettably few instances in which actual protocols have been recorded. A worker may describe a new technique in great detail, but the result of using it is represented by a graph, (sometimes bearing practically no indication of the magnitudes involved) on which the points through which the Standard curve is drawn are shown as a series of crosses. From this, by measurement and calculation, one can laboriously deduce approximately what the original mean responses and doses may have been; but no idea of the agreement between replicates or of the validity of the results can be obtained, since the actual titrations are not shown and no typical Test Preparation is included. This neglect of the numerical, as opposed to the experimental, details of new analytical work in general is to be deplored. It is as important to be able to check the first as the second—in fact, more so; few will wish to learn about a new and elegant method of obtaining inaccurate results. I strongly urge that no publication of a new analytical technique in this field at any rate should be regarded as complete or accepted for publication unless it includes the protocols of a typical assay.

Examination of the few published dose - response curves from which reasonably accurate estimates of the original data could be made eventually showed that when the logarithms of both the doses *and the responses* were plotted against each other the resulting points were fitted reasonably well by a straight line, at least over a certain range. The enquiry was then extended to a number of assays for both amino-acids and vitamins, performed in four different laboratories, the protocols of which were made available to me by the most willing co-operation of the workers concerned. The linear "log - log" relationship, as it may be called for brevity, was found to hold good almost without exception, though not always over the whole range of doses used. This, of course, is to be expected; the linear relationship found in riboflavine and nicotinic acid assays holds only up to a titration of 8 to 9 ml. of 0·1 N sodium hydroxide at most, in terms of response (which is the correct criterion[6]), whereas the doses used in amino-acid assays are usually chosen to give a maximum response of 12 to 13 ml. or even more.

A typical instance of an assay in which the log - log relationship holds is shown in Fig. 1, an assay of threonine by means of *S. fæcalis*, as described by Barton-Wright[4] and performed by him. The curves obtained by plotting dose against response, for both the Standard and the Test Preparations, are transformed into two straight lines when log-dose is plotted against log-response. The range of linearity is at least from 20 to 80 μg. in terms of dose, or from 3·55 to 9·05 ml. of 0·1 N alkali in terms of response.

The log - log relationship appears to fit nearly all the assays on which it has so far been tried. Table I summarises the position. The technique used in these assays is by no means standardised: the test organisms used differ from worker to worker; even where the same organism is used the basal medium may differ. The variations in slope and in range shown in the table are thus not at all surprising.

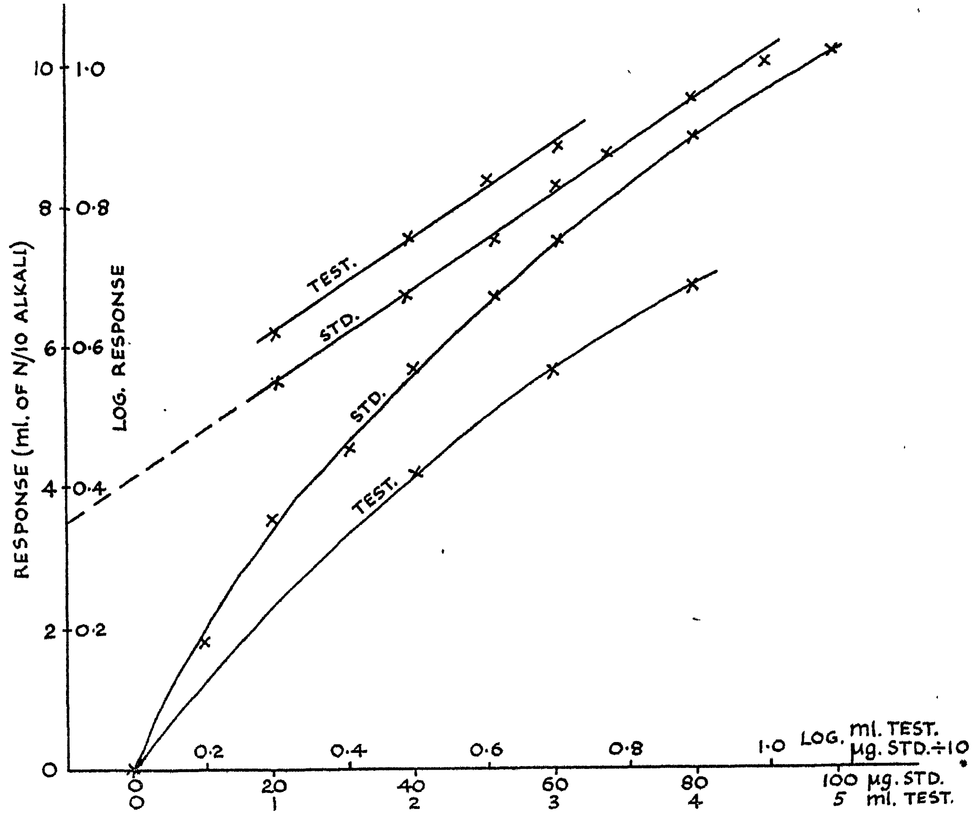


Fig. 1. Assay of Threonine with *S. fæcalis*.

Certain entries call for special mention. The assay of glutamic acid is biochemically unusual, since it appears that the substance utilised by the test organism is not glutamic acid but glutamine,[7] and this results in the dose - response curve being initially almost horizontal, restricting the range of linearity, while the next part of the curve is steeper than usual. The technique of this assay is not satisfactory. The assay of proline, as conducted in the Glaxo Laboratories, was different from all the other assays in giving a linear relationship between log-dose and the response itself; and it is interesting that the basal medium used by them for this assay differs from that used by them for all the other amino-acids in the table.

One or two of the assays probably have a greater range of linearity than that shown because the doses were not well chosen to test the point (see below). For example, in the assay of histidine referred to, the lowest dose used was 10 μg. and the corresponding mean response was 6·15 ml. Had doses of 5 μg. and even less been used it is quite probable that the range of linearity would have been extended usefully downwards. This applies also to other assays.

The computation of the mean result and its standard error, and the examination of the validity of the assay, have been fully described elsewhere, notably by Irwin,[8] for all assays in which either the response itself or some function thereof (the response metameter, as Bacharach[9] has called it) is linearly related to the log-dose. But for those who are not familiar with the principles involved, a brief summary may be useful.

(*a*) If the Standard points lie satisfactorily on a straight line between certain limits of response, so should the Test points lie within the same limits. (Any experimental observations outside these limits are best ignored in the subsequent calculations.) Significant curvature in the Test line would render the validity of the assay suspect. Moreover, the Test line should be parallel to the Standard line, and significant lack of parallelism is again evidence of invalidity.

TABLE I

ASSAYS FOUND TO BE MADE LINEAR BY THE "LOG - LOG" TRANSFORMATION

Substance assayed	Reference*	Test organism	$\frac{\text{Upper limit}}{\text{Lower limit}}$ of response	Slope	Remarks
Amino-acids:					
Arginine ..	B	*Strep. fæcalis*	2·98	0·82	
	G	*Strep. fæcalis*	2·02	0·52	
Cystine ..	B	*Lact. arabinosus*	2·26	0·45	
	G	*Leuc. mesenteroides*	2·02	0·33	
	V	*Lact. arabinosus*	2·32	0·78	
Glutamic acid ..	G	*Leuc. mesenteroides*	1·87	1·16	Linearity doubtful
Histidine ..	G	*Strep. fæcalis*	1·67	0·36	Lower limit probably too high
*Iso*leucine ..	B	*Lact. arabinosus*	3·18	0·65	
	G	*Leuc. mesenteroides*	2·25	0·58	
	V	*Lact. arabinosus*	2·52	0·85	
Leucine	G	*Strep. fæcalis*	3·37	0·69	
	V	*Lact. arabinosus*	2·24	0·76	
Lysine	B	*Leuc. mesenteroides*	4·97	0·70	
	G	*Strep. fæcalis*	2·86	0·58	
	V	*Leuc. mesenteroides*	2·99	0·69	
Methionine ..	B	*Leuc. mesenteroides*	2·90	0·79	
	G	*Leuc. mesenteroides*	2·01	0·42	
	R	*Lact. arabinosus*	3·57	0·74	
Phenylalanine ..	B	*Leuc. mesenteroides*	3·73	0·73	
	G	*Leuc. mesenteroides*	3·07	0·46	
Proline	G	*Leuc. mesenteroides*	Linear, log-dose against *response* 3·3 to 14·05 ml. of 0·1 *N*		
Threonine ..	B	*Strep. fæcalis*	2·55	0·68	
	G	*Strep. fæcalis*	3·13	0·72	
Tryptophan ..	B	*Lact. arabinosus*	7·4	0·76	
	G	*Lact. arabinosus*	6·08	0·84	
	V	*Lact. arabinosus*	2·87	0·67	Lower limit probably too high
Tyrosine ..	G	*Leuc. mesenteroides*	3·20	0·83	
	Gu	*Lact. delbrückii*	2·61	0·72	
Valine	G	*Strep. fæcalis*	1·72	0·60	Linearity doubtful
Vitamins:					
Biotin	D	*Lact. arabinosus*	2·83	0·45	
	Vl	*Lact. arabinosus*	2·74	0·71	
Folic acid ..	J	*Lact. helveticus*	2·13	0·58	
Riboflavine ..	Ro	*Lact. helveticus*	3·58	0·56	

*REFERENCES

B = Dr. E. C. Barton-Wright.
D = The Distillers' Co., Ltd.
G = Glaxo Laboratories, Ltd.
Gu = Gunness *et al.*, *J. Biol. Chem.*, 1946, **163**, 159.
J = Johnson, B. C., *Ibid.*, 1946, **163**, 255.
Ro = Roberts, E. C., and Snell, E. E., *Ibid.*, 1946, **163**, 499.
R = Riesen *et al.*, *Ibid.*, 1946, **165**, 347.
V = Vitamins, Ltd.
Vl = Virol, Ltd.

(*b*) Assuming that the lines are straight and parallel between approximately the same limits of response, the best and quickest way of computing the result is to measure the *horizontal* distance from the Test line to the Standard line. When this is added to the logarithm of the potency of the Standard Preparation, the sum is the logarithm of the potency of the Test Preparation. For example, in Fig. 2, which represents an assay of lysine by Mr. S. A. Price, of Vitamins, Ltd., using *Leuconostoc mesenteroides*, the horizontal distance along the line "log-response = 0·75" is 0·765 − 0·421 = + 0·344; (note that the Standard figure must be taken first). The Standard Preparation in this assay was pure lysine, and the unit was 10 µg., so that its logarithm is 1·000. Hence 1 unit of the Test extract, which here is 1 ml., contains antilogarithm 1·344 = 22·1 µg. of lysine. Knowing the dilution of the Test extract, one can readily calculate the potency of the original Test substance. In this assay the lines are not quite parallel, and the measurement was taken near the mid-point. A better procedure, which also gives an indication of the validity of the assay, is to make the measurement near the extreme limits of linearity and examine the agreement between the two resulting estimates of potencies. If agreement is good, the mean is taken as the answer. For log-responses of 0·5 and 0·9, the corresponding estimates of the Test extract potency in Fig. 2 are 21·5 and 22·5 µg. per ml., with a mean of 22·0. The extremes are within 3 per cent. of the mean, which is satisfactory.

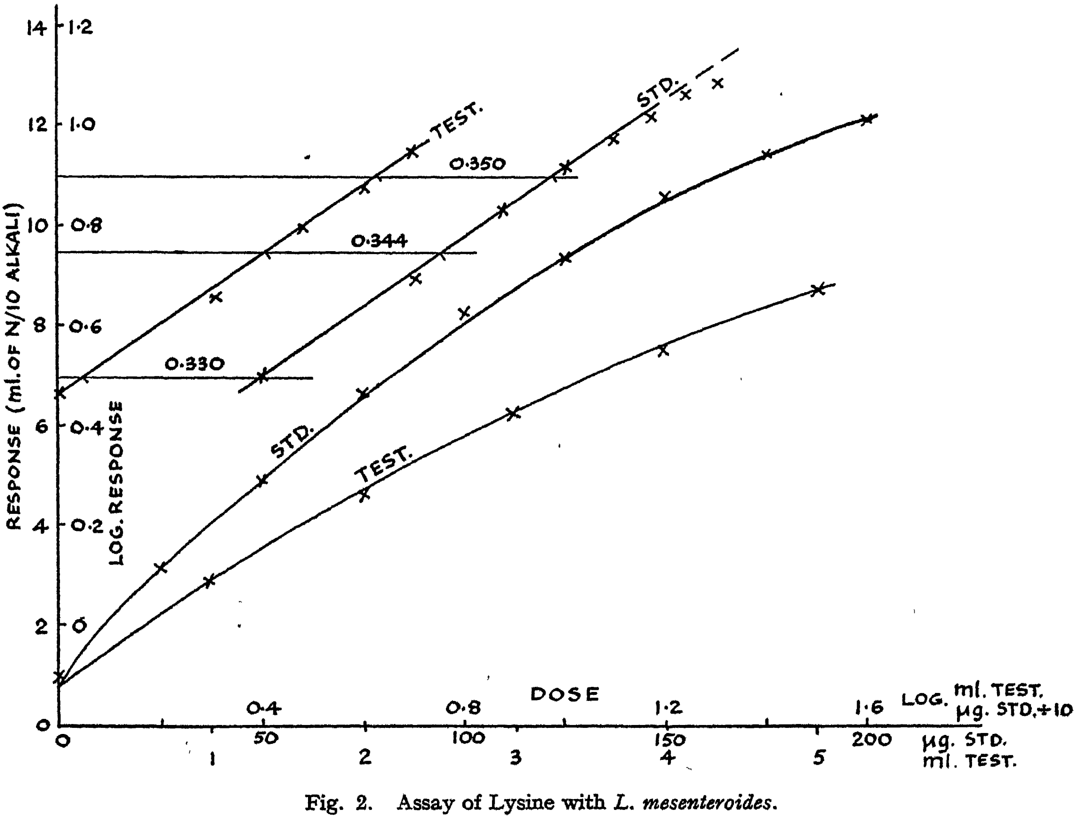


Fig. 2. Assay of Lysine with *L. mesenteroides*.

When the lines are not perfectly parallel the theoretically best estimate of the result is obtained only by a rather lengthy arithmetical process, because the experimental designs at present employed lead to unequal spacing of the dose-levels when plotted on a logarithmic scale and the number of Test doses between the limits of linearity is hardly ever equal to the number of standard doses between the same limits. This means that the slopes of the two lines will usually be determined with unequal precision. Moreover, converting the responses into logarithms usually results in a decrease in variance with increasing dose, which gives more weight to the observations at the higher dose-levels. Consequently, the top half of each line has usually more weight than the bottom half and the weighted mean of the results is not exactly at the mid-point. Mr. D. J. Finney, in correspondence on this subject, gives it as his opinion that "usually the two methods [the graphical and the full statistical] would lead to estimates of potency in very close agreement, but there is a possibility that your method if adopted generally and uncritically might fail to point out invalid assays, and might occasionally give a misleading potency estimate. Further investigation of this point, especially by the detailed study of numerical examples, is needed as a research project." If an estimate of the confidence limits of the result, or a quantitative test of validity, is needed, then the appropriate calculations, lengthy though they are, will have to be carried out in full in any event. The moral is that the faults of the design should be eliminated as suggested below, which would have the double advantage of simplifying the arithmetical work if the full statistical calculations are carried out and also of reducing risk of error if the quick graphical method is employed. The technique of the statistical method is not new and will not be described here; reference may usefully be made to the paper by Irwin[8] already mentioned.

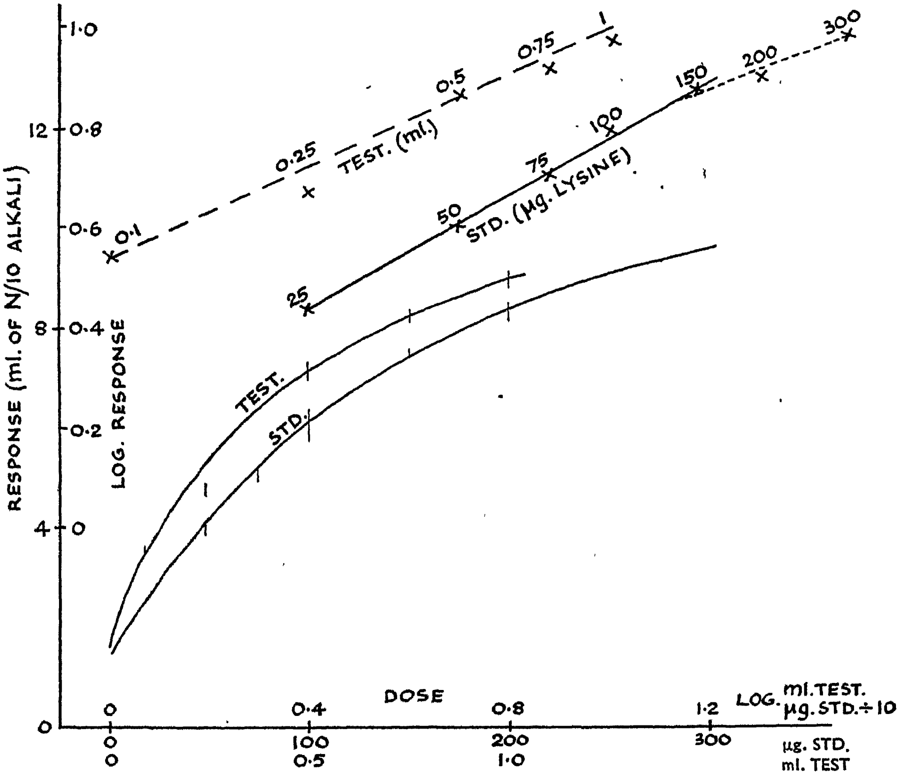


Fig. 3. Assay of Lysine with *S. fæcalis*.

If the "log - log" relationship is found as a matter of experience to hold over a reasonable range, then certain suggestions immediately follow for improving the design and simplifying the computation of the assay. In the first place, the position of the line will be fixed most precisely for a given number of doses, if not they but their logarithms are equally spaced, which means that the doses both of the Standard Preparation and of the Test Preparation should be in geometrical progression. In the example shown in Fig. 2, for instance, the doses used increase uniformly by 25 μg. or 1 ml. as the case may be. This is a natural arrangement, but the result is that the experimental points on the log - log lines are crowded together at the upper end of each line, which is thus better defined than the lower. Had the doses chosen for the Standard Preparation been 25, 35, 50, 70, 100, 140 μg.—a series in which each is $\sqrt{2}$ times the preceding one—the resulting points would have been separated by a constant difference of 0·15 in terms of log-doses. Similarly, a good series of Test Preparation doses would be 0·5, 1, 2 and 4 ml., each being twice the preceding one. It would be even better if the number of doses, as well as the ratio of consecutive doses, were the same for both lines, for then the design is symmetrical and the computations are simple. As usual, the Test doses should be so chosen as to be equivalent as far as possible to the Standard doses in their content of the factors being assayed.

Once it is decided to adopt the "log - log" transformation as a preliminary to evaluating the result, observations should be confined as far as possible to that part of the dose - response curve which is rendered linear by the transformation. In Fig. 3, for instance (an assay of lysine by means of *S. fæcalis*), the upper two observations on each of the Standard and Test curves are above the linear portion. This leaves observations at only three dose-levels on which to base the Test line, and makes it difficult to fix its position, particularly if (as in this assay) the "fit" is not good. Four or five equally spaced points on each line should be secured if possible; in this example it would have been practicable to use Standard doses of 10, 20, 40, 80, and 160 μg., with Test doses of 0·1, 0·2, 0·4, 0·8, and 1·6 ml. Probably the lowest Standard dose and the highest Test dose would have been found to be "off the line"; that would have left a symmetrical design with 4 equidistant points on each line. The resulting computations would then have been simple. This is the kind of design to aim at, at any rate; with any assay performed regularly according to a standardised technique, experience should soon show how to secure it. The *extreme* limits of linearity and the slope will of course vary, not only between laboratories, but even between assays by the same worker; yet the slopes of all the assays in Table I are very much larger than the standard error (usually of the order of 0·01 to 0·03 in these assays), which means that in every instance the fiducial limits of the result should be sufficiently close for all practical purposes if the assay is valid at all, while safe working limits of dosage within which every assay will be linear can be determined by experience for each amino-acid or other nutrient factor separately.

It has been stated above that the "log - log" transformation has the effect in most instances of producing an error variance which decreases with increasing dose. This may be deplorable in theory; in practice, owing to the good agreement between replicates and the excellent approximation to linearity which appears usually to obtain in these assays, the effect on either the accuracy or the precision of the result is unlikely to be large. This point, however, warrants further examination.

While the ratio of the slope to its standard error is the criterion of the inherent precision of an assay technique, the sensitivity with which invalidity will be detected depends on the range of linearity in terms of log-response, which corresponds to the ratio of the upper to the lower limit of linearity in terms of the response itself. The fourth column of Table I shows that there is considerable variation in this respect between assays, but, as previously stated, further work would probably show that the range of linearity can often be extended.

One or two points remain to be made. First, the "log - log" transformation, which is not at all original, is purely an empirical device for expediting the extraction of information from an assay; it is not suggested that there is any profound biochemical significance in the fact that many assays are "linearised" by its use, or that an assay which does not conform to this generalisation is any the worse for it. Secondly, the suggestions made in this paper for improving the design of "log - log" assays are put forward only in the hope that they may prove helpful to those workers who use such assays primarily to obtain quantitative information about the chemical analysis of foods and the like. They are not applicable to the research worker who is investigating the biochemical processes involved, and whose needs are quite different, as Finney[10] has pointed out. Finally, nothing in what I have said must be taken to imply any criticism of the assay protocols on which this paper is based; they are in the main as well constructed as was possible in the absence of any information about a "linearising" transformation or the range over which such a transformation holds.

I am greatly indebted to Dr. E. C. Barton-Wright, to Mr. S. A. Price of the Research Laboratories, Vitamins Ltd., to the Research Division of Glaxo Laboratories Ltd., and to Dr. E. R. Dawson of The Distillers Company Ltd., for their kindness in allowing me to see and use their experimental data, as well as to Mr. D. J. Finney for advice on statistical theory.

SUMMARY

In microbiological assays of many specific nutrients the relationship between dose and response is not linear. It is now found that when the logarithm of the dose is plotted against the logarithm of the response the resulting points are usually well fitted by a straight line over a reasonable range. This makes it possible to compute the best estimate of the result and its standard error, and to test quantitatively the validity of the assay, by methods which accord with accepted statistical principles. The assay can be made more efficient and the calculations simplified by modifying the experimental design. The doses of both the Standard and Test Preparations should be in geometrical, not arithmetical, progression, and the assay should be as far as possible symmetrical in the number and spacing of the doses,

which should be confined to that part of the dose - response curve which the "log - log" transformation renders linear. Several questions that arise from these considerations need further investigation.

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3. Report on the Microbiological Assay of Riboflavine and Nicotinic Acid, ANALYST, 1946, **71**, 397.
4. Barton-Wright, E. C., *Ibid.*, 1946, **71**, 267.
5. Price, S. A., *Nature*, 1945, **156**, 171.
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7. Hac, L. R., Snell, E. E., and Williams, R. J., *J. Biol. Chem.*, 1945, **159**, 273.
8. Irwin, J. O., *J. Hygiene*, 1943, **43**, 121.
9. Bacharach, A. L., Coates, M. E., and Middleton, T. R., *Biochem. J.*, 1942, **36**, 407.
10. Finney, D. J., ANALYST, 1946, **71**, 14.

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DISCUSSION

Mr. S. A. PRICE said that the use of closely-spaced doses in geometrical progression was quite practicable, and was in fact normal in serological testing, when doses each of which was 110 per cent. of the preceding one were commonly used. He asked whether the log - log transformation had been found applicable to a large proportion of the assays examined. If assays were designed on the lines suggested, then those occasional assays that did not conform to the log - log relationship would be difficult to evaluate by the direct-reading method.

Dr. E. R. DAWSON reported that he had found the log - log relationship to hold over a wide dose-range for a long series of biotin and pantothenic acid assays using *L. arabinosus*. He was now trying in his laboratories a four-point assay design with a dose-ratio of 4 for both standard and test substance. The log - log relationship could not be expected to be linear at very low dose-levels in assays which do not give a blank of zero. Had Dr. Wood considered this point?

Mr. E. C. FIELLER pointed out that Dr. Wood's suggestion that the linear range might be usefully extended downwards in certain instances by using smaller doses might lead to trouble if carried too far, since the variance of the response after logarithmic transformation must tend to increase with decreasing response, and would become excessively high below a certain value of response.

Dr. WOOD, replying to Mr. Price, said that Dr. Dawson's experience indicated that the log - log relationship, if it held at all for a given type of assay, was consistently applicable in a large series. Moreover, if an occasional assay were exceptional, the direct-reading method could still be used provided that there were three or more doses of each preparation. It was true, as Dr. Dawson had said, that the log - log transformation could not be expected to "linearise" an assay down to very low dose-levels, and indeed it would be dangerous to go too far downwards for the reason advanced by Mr. Fieller. Each worker should find out for himself, by trial and error, the maximum safe range of doses that could be used in a particular assay technique.

Electrometric Analysis

(*The following three papers were read at the Joint Meeting of the Physical Methods Group with the Cardiff and District Section of the Royal Institute of Chemistry and the South Wales Section of the Society of Chemical Industry, at Cardiff, on Friday, October 11th,* 1946.)

Recent Developments in Apparatus for *p*H Measurements and Electro-titrations

BY A. D. ELMSLY LAUCHLAN

THE use of the thermionic valve in a *p*H measuring device has enabled manufacturers to supply an instrument so robust and simple to use that even unskilled and semi-skilled workers are now able to make precision measurements.

Ability to use a well designed *p*H meter, however, does not always go with the careful technique necessary for proper use of chemical apparatus, and much trouble and annoyance have been the lot of many when the glass electrode has been broken owing to a moment's carelessness. It has been the aim of the instrument maker to produce electrodes as robust as the associated instrument, and to this end thicker and thicker glass electrodes have been produced, so that now if the modern glass electrode is knocked on the bench the stem is more likely to be broken than the bulb.

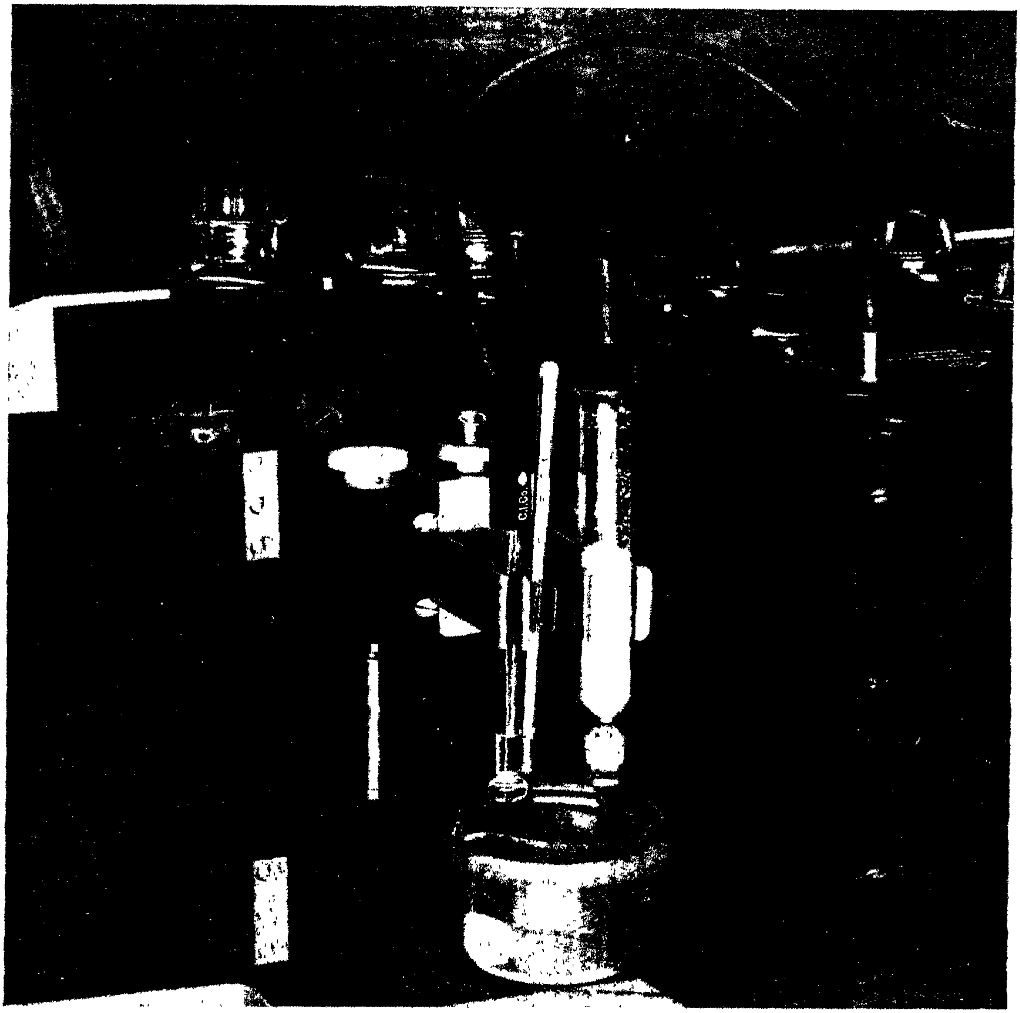

Fig. 1. *p*H Meter with screened lead from glass electrode to earthed instrument panel.

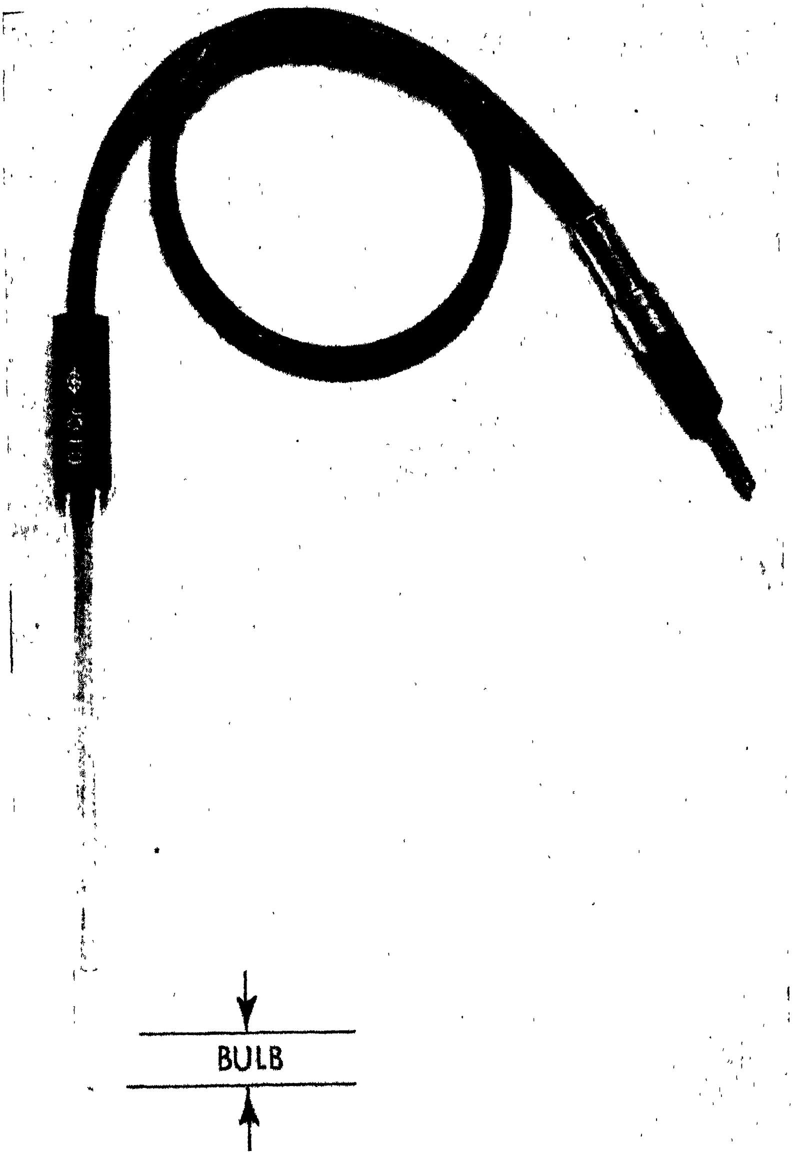

Fig. 2. Spear Type Glass Electrode. Note long insulation (black) between screen and tip of plug.

It should be appreciated that the difficulties of making glass electrodes stronger have been not in the actual blowing of the bulb but in arranging conditions so that the very minute amount of energy available can be applied to the measuring circuit without appreciable loss.

When it is remembered that the resistance of a glass electrode is of the order of 150 to 200 megohms, it will be understood that the leakage permissible must be extremely small in order to obtain an accuracy of 0·1 per cent.; a leakage resistance of 1000 megohms will lower the efficiency of the system by about 8–9 per cent.

Improvement in insulation alone will not make the measuring instrument read correctly if the valve is not suitably chosen or arranged to have the correct condition in which the grid takes a negligible current, *i.e.*, 10^{-12} amperes at the most. Most modern instruments are no longer fitted with the electrometer valve, which did not lend itself to sub-panel mounting and, moreover, was somewhat sensitive to shock. The great advantage of the electrometer valve was its very high grid resistance, but it is now possible to use some of the more conventional types of valves and so adjust their working conditions that they act as electrometers or very nearly so. The use of these smaller valves has given greater stability to mechanical shock, enabling a much steadier electrical zero to be maintained, and has allowed the manufacturer to produce a neater instrument.

When the grid of a valve is connected to a source of potential having a high internal resistance, *e.g.*, a glass electrode, the circuit becomes very sensitive to stray electrostatic charges such as arise when the connecting cable of the electrode is touched or moved, and this gives rise to much annoyance by causing the galvanometer needle to wander.

This trouble can be overcome only by shielding the circuit and connecting the shield to earth, but this shielding must extend from around the valve right up to the electrode and much ingenuity has been exercised in devising a simple connection for the glass electrode, suitably insulated and shielded so that the user has the benefit of high insulation and correct shielding (Figs. 1 and 2). This problem becomes even more acute when long lengths of cable, often up to 200 feet, have to be used. The problem occurs more with industrial recording instruments than with laboratory apparatus, but that it has been successfully overcome indicates that the principles employed are right.

These apparently small changes have in reality done much to permit the use of stronger electrodes, which are, at the same time, less disturbed by stray electrostatic charges.

There are now appearing in England direct-reading *p*H meters or rather indicators, which show at a glance the actual *p*H value or any change occurring in it without any further manipulation of the instrument controls. These instruments undoubtedly have a great advantage over the type that requires balancing by hand, but it must be remembered that their accuracy will, in general, not be as good as that of a null-method instrument, since the deflection of the pointer is determined by the characteristic of the valve, which may change. A further point is that the limited scale length, even with a double scale of *p*H 0 to 7 and 7 to 14, one cannot read accurately to 0·01 *p*H, which is quite often required.

The null-point instrument and the direct-reading indicator really fill two separate requirements, the first giving high accuracy where speed of operation is not quite so important, the second giving a somewhat lower accuracy of ± 0·05 *p*H, which for very many problems is quite sufficient. The popularity of the direct reading indicator in the U.S.A. is an indication of its usefulness.

The so-called "magic eye" indicator has not so far replaced the galvanometer in English *p*H meters, as has already happened in some instances in America, probably because the range of valves suitable for convenient incorporation in the instrument is not yet readily available here.

From an examination of these indicators it does not seem easy to judge the balance point to an accuracy of 0·01 *p*H, possibly because there is no real fiducial point from which to measure the change; also the general sensitivity seems to be lower than that of a galvanometer. The cost of the electronic indicator and its associated equipment is not likely to prove less than that of a good galvanometer, and while they may be less sensitive to mechanical damage a well made galvanometer can also stand up to rough usage.

It is, moreover, interesting to see that many American manufacturers are still using the galvanometer as the sensitive detector, so it may be a matter of doubt as to whether the electronic form of detector can strictly come under the heading of improvements.

Turning now to the glass electrode itself it has been found that the best results are obtained if the glass is made from very pure materials so that impurities, chiefly alumina are kept as low in amount as possible, generally 0·25 per cent. The production of pure glass enables an electrode to be made which will give theoretical results over a wide range of *p*H. The use of the pure glass enables us to make very small electrodes, which are yet sufficiently conducting to give theoretical results over the same range as the normal electrode. These small bulbs, which may be only 2·5 mm. in diameter, are exceedingly useful for examining small quantities of material or for carrying out electro-titrations on a few drops of liquid, such as might be used in a Conway vessel. The small electrodes also find considerable use in medical and dental work, such as the examination of saliva in the pockets formed between the cheek and the gums.[1] When fitted with a correctly shielded lead these electrodes give very steady potentials on a *p*H meter.

Probably the most outstanding improvement in glass electrode performance is the production of a glass which is less sensitive to sodium ions and enables accurate measurements to be made up to *p*H 14 and in solutions more concentrated than 1 Normal. The improvement in performance can be judged from the fact that the normal glass electrode has a sodium error of about 0·6 *p*H at *p*H 12 whereas the new glass reduces this error to zero even on a 1 Normal solution of sodium ions (Fig. 3). While it is necessary to know the sodium ion concentration in order to apply the appropriate correction to obtain the best results, the error introduced by an uncertainty of the ion concentration is not nearly so serious. The general accuracy of this new glass electrode is about 0·02 *p*H. For those who are not acquainted with this new electrode it may not be out of place to point out that, while the electrode can give accurate results in the acid range below about *p*H 9, it offers no advantage at all over the normal electrode, and exposure to an acid medium for any length of time ages the glass so that its response in the higher range of *p*H values is spoiled. The new glass can be made into bulbs of the usual shape and size and, apart from any special identification mark put on by the makers, cannot be distinguished from the normal type.

The reference electrode to be used with glass electrodes has undergone but little change except that for some purposes, such as medical, the usual ground sleeve type of junction has been replaced by a sintered plug, which can, if need be, take the form of a fine pointed tube, so as to occupy only a small part of the space available when micro tests are being made, or where it is necessary to introduce the cell connection into cavities in the body, *e.g.*, the mouth.

The general trend of electrode structure is to have the electrodes stronger and smaller, and in the future it may well be possible to reduce the present sizes even more.

In the field of recording and control of *p*H values, the general principles which have produced improvements in manually operated instruments also hold, and the changes that have enabled closer control to be maintained in batch and flow processes are applicable to all types of controllers, but these are rather outside the present subject.

In spite of the fact that the literature on potentiometric titrations extends back over a considerable number of years, there does not seem to have been any great application of the methods until the last war. Possibly the need for a large number of routine analyses to be carried out by semi-skilled workers called for a simpler apparatus than had been available before. The earlier forms of electro-titration apparatus, developed mainly by Dr. Sand, were rather akin to some of the earlier *p*H meters and did not lend themselves to easy operation or understanding by the semi-trained. The use of the hydrogen electrode, with all its attendant troubles, no doubt accounted for their lack of popularity.

The rapid increase in the pace of war production made the demand for a simpler electro-titration apparatus more urgent. The experience gained in the development of *p*H meters was now directed to improving an old but otherwise useful apparatus, so that we now have something which bears but little resemblance to the collection of parts, which formed the prototype apparatus.

The modern sensitive galvanometer and the "magic eye"are now competing for the place of detector, and here at any rate the "magic eye" seems to have an advantage since it can be mounted on the burette stand. The slightly lower sensitivity of the "magic eye" detector is not so important here as, unless the conditions of the reaction going on in the beaker are not well chosen, there should be a reasonably large change of potential at the end-point. It may, perhaps, be permitted to remind the user that no matter how good the instrument may be, the best results cannot be obtained unless the conditions of the chemical reaction are properly arranged, for the indicator electrode can only pass on to the detector the results of the change that it experiences in the reaction.

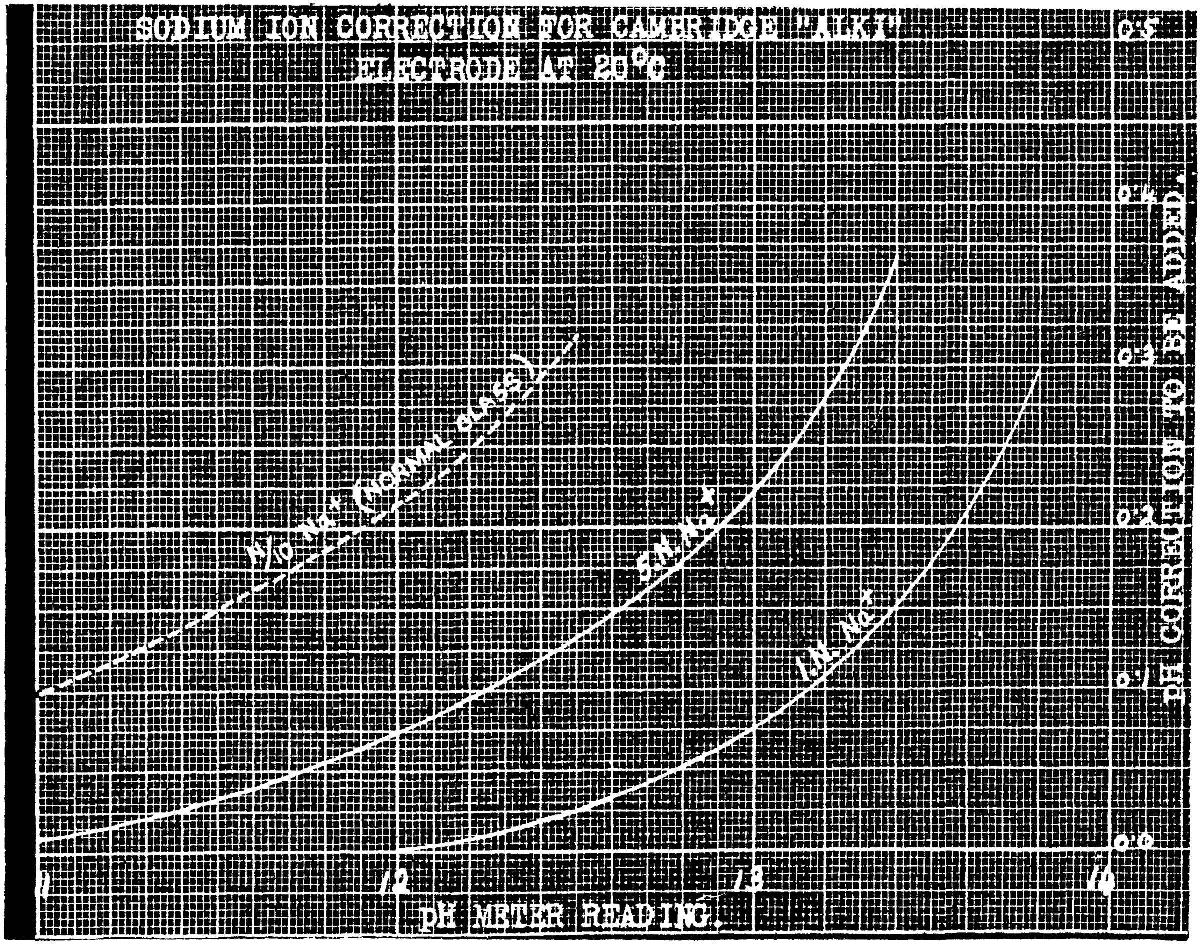

FIG. 3

Curve showing corrections for high pH glass electrode. Dotted curve shows error of normal glass electrode.

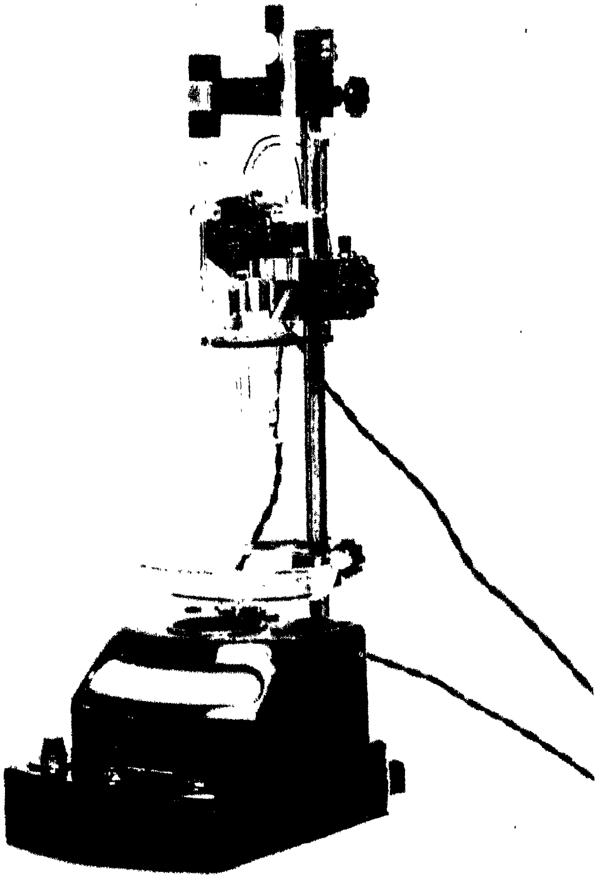

Fig. 4. Potentiometric Titration Apparatus fitted with galvanometer.

Here again English and American practice seems to offer fairly equally the two forms of detector, so that it would appear that opinion as to the better type is still undecided.

The electro-titration apparatus (Fig. 4) now consists of a stand carrying the burettes and possibly an indicator of the end-point and a small box in which are placed the potentiometer and any amplifier for operating the detector. The potentiometer may be calibrated in millivolts or left uncalibrated; it is not very important which system is used, as in general it is only necessary to balance out the initial potential developed by the electrodes and then note by means of the movement of the detector the change in this potential at the end-point.

The instruments are battery- or mains-operated, depending on the use of a galvanometer or "magic eye" detector, and there is little to choose between them as a matter of convenience.

While the uses to which the electro-titration apparatus may be applied hardly come under the heading of "recent developments," it may not be out of place to mention some of the newer applications.

The beautiful method devised by Foulk and Bawden[2] in the U.S.A. and known as the "dead-stop end-point," uses two noble metal electrodes which are polarised by the passage of a current until an excess of one reagent depolarises them and causes a large increase in the current through the detector; alternatively the current can be reduced almost to zero by addition of a reagent that reacts with and removes the depolariser.

The titration of thiosulphate or sodium arsenite with iodine produces the first condition and the inverse operation provides the second. The system has, therefore, a very wide scope of application and has been used for the determination of oxygen in turbine condensate[3] and for the estimation of moisture by the Karl Fischer method,[4] in which iodine in dry pyridine, saturated with sulphur dioxide, is caused to react with the water in the test material.

It is a matter of regret that so far no chemical manufacturer has put on the market the necessary reagents, but from enquiries made this deficiency is shortly to be remedied. Admittedly it is a somewhat revolutionary method of determining moisture but the oven drying method is not above reproach.

An examination of current literature will provide a far more suitable source of information on electrometric titration than can be compiled in a short paper of this description, since it ranges from the determination of vitamins to steel analysis, but it may not be out of place to point out that the elegant method of Foulk and Bawden can often be applied more satisfactorily than that in which a change of potential takes place, *e.g.*, reactions involving the use of potassium permanganate.

Conductometric titrations do not seem even yet to have become so widely used as the potentiometric method and its modifications, possibly because more trouble is caused by the comparatively large temperature coefficient of resistance of a solution, which requires much greater care to be taken to ensure constancy of temperature during the experiment.

There are available now very satisfactory conductance bridges operating at about 1000 cycles per second, fitted with a telephone and a galvanometer or a "magic eye" as detector of balance, so there does not seem to be any obvious reason why this analytical method has not progressed like the potentiometric.

It is perhaps not too much to expect that the future will bring, and at no very distant date, further improvements in the instruments themselves and also in the glass electrodes, the working of which we are as yet only beginning to understand. The recent advances have opened up a field of thought which may well provide some very interesting results.

Thanks are due to the Directors of the Cambridge Instrument Co., Ltd., for permission to make use of data obtained in their work and for the loan of instruments.

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Some Applications of Electrometric Methods in Analysis

BY R. J. CARTER

THIS paper endeavours to review a few of the more useful electrometric methods available for use in quantitative analysis and also to observe some new trends in analytical research prompted by those methods. The scope of the paper is necessarily restricted and many examples, including the whole group of conductometric methods, are consequently omitted. Reference will be confined to potentiometric titrations, divided into sub-groups of acid - alkali, precipitation and oxidation - reduction types, concluding with some considerable reference to the mechanism and application of the Karl Fischer reagent electrometric method for the determination of small amounts of water.

Probably the widest review with bibliography of potentiometric titrations is that given by Furman,[1] who gives references to published work over the last twenty years, and has himself made many valuable contributions. For detailed methods of many types, the analyst is recommended to refer to that review.

ACID - ALKALI TITRATIONS

The relative ultimate accuracy of indicator and potentiometric methods largely depends upon the appearance of inflection in the neutralisation curve. In general, the latter is governed by the magnitude of the product K.C relative to the ionisation constant of the solvent, where K is the ionisation constant of the weak acid or base and C the concentration.[2] In aqueous solutions no inflection appears unless K.C is greater than 27 K_w,* and for practical purposes K.C should be greater than 10^{-10}. Where the inflection is not sharp it may still be possible to estimate the end-point with fair precision, as the point where the second differential changes sign; if the reagent has been added in equal increments this may be calculated from differences between successive titration values; alternatively it may be found by examination of the graphs of e.m.f. against volume of reagent.

Apart from the obvious application to titrations of coloured solutions, the use of the potentiometric method is of value where the indicator end-point is evanescent, such as with weakly dissociated acids or bases, a practical example being heavily bodied vegetable oils. The reaction towards the end of the titration may be slow, requiring some time for a steady e.m.f. reading at each addition, but the graphical calculation of the equivalence point will be accurate and will give a result truer than that obtained with an indicator.

The titration of materials soluble only in non-aqueous solvents may be carried out by rendering the solutions conductive, *e.g.*, by addition of lithium chloride, or by use of selected solvents. Titrations in non-aqueous media in the latter category have been described by Lykken, Porter, Ruliffson and Tuemmler[3]; one of the criteria required of the selected solvent or solvent blend is that it should have properties such that dissolved acidic materials, with dissociation constants greater than 10^{-7} in water, will ionise sufficiently to permit neutralisation by an equivalent quantity of alcoholic strong base solution. It should be sufficiently conductive to allow only momentary accumulation of electrostatic charges when a low resistance reference electrode is immersed in it. Lykken and his co-workers titrate in a solvent blend of equal parts of benzene and *iso*propyl alcohol containing 1 per cent. of water and use a glass - calomel electrode system. (To avoid using the *p*H unit when referring to non-aqueous solutions, the term cG unit was selected and is given in volts.[4] It was found that most strong acids in the non-aqueous medium gave an inflection near a cG value of 0·236 volts, equivalent to a *p*H of 4·0, and weak acids one at 0·650 volts, equivalent to a *p*H of 11·0. Where definite breaks or inflections are not apparent the titrations to cG 0·236 and 0·650 volts were taken as equivalent to strong and total acids respectively.)

The use of a titration curve with more than one inflection to detect different acids and bases present together is well known. It is possible to determine hydrochloric acid in presence of either formic or acetic acid, and these in turn are distinguishable from weaker acidic compounds, such as thiophenol.

Combined acids may be determined by saponification treatment; the differences between titrations for sample and blank to reach a cG of 0·650 volts is a measure of the total free and combined acidic constituents, *i.e.*, the saponification number. The corresponding differences to reach the lower cG value of 0·236 volts is a measure of the free and combined strong acid constituents only.

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* K_w = dissociation constant of water.

A method has been devised whereby saponification numbers of fats and oils can be determined and the need for a blank titration is eliminated.[5] The potentiometric titration curve obtained after saponifying the oil and titrating with acid shows two breaks, the acid added between the two points of inflection being used for the reaction

$$RCOO' + K^{\cdot} + H^{\cdot} + Cl' \longrightarrow RCOOH + K^{\cdot} + Cl'.$$

This is equivalent to the saponification number of the oil, so that a blank titration is not required. It may be that the slope of the graph at the second break is not steep enough to determine accurately. The slope is proportional to $(K.L.V)^{-\frac{1}{2}}$ where K is the ionisation constant of the fatty acid, L is the number of millimoles of fatty acid in solution at the equivalence point and V is the total volume of solution. An increase in slope therefore can be brought about by decreasing L, namely by adding benzene to extract fatty acid into the benzene phase. Inflection points are located by calculating second differentials in the usual way.

The use of non-aqueous solvents to differentiate further the ionisation characteristics of two or more solutes represents a new method of approach in analytical research. By its means it becomes possible to obtain a differential titration which may be unsatisfactory or impossible in aqueous medium. Various workers have investigated the use of acetone - water mixtures, alcohols, benzene and Cellosolves. Lykken and others[3] showed that in the selected blend of *iso*propyl alcohol and benzene the relative acid or base strengths of the members of a series of acids or bases are in the same order as in water, and also showed graphically that there is a relationship between the relative strengths deduced from titrations and the pK_a* values in water.

It is also possible to show by varying the media to different degrees of aqueous content or water-like properties, that a weak acid shows a higher degree of ionisation in water than in non-aqueous media. Strong acids do not follow a strictly regular trend and appear to show a maximum degree of ionisation in solvents that are intermediate in water-like properties. Practically it means that a non-aqueous solvent medium may be chosen in which it will be easiest to distinguish between strong acids whose pK_a (H_2O) values are in a close range.

PRECIPITATION AND OXIDATION - REDUCTION TYPE TITRATIONS

It is not intended to list the applications of these types of titrations—the theory and use of them are too well known. Some contributions by different workers in recent years are of considerable interest, however, and their value may be the more apparent when the findings are applied to other problems.

The use of electrolyte bridges in potentiometric titrations where a bimetallic electrode system cannot be employed often has the disadvantage of the risk of contaminating the test solution. Heintze[6] in 1934 suggested that the glass electrode might be used as a reference electrode as opposed to its usual function as an indicator electrode. It has been established that the glass electrode behaves as a satisfactory reference in potentiometric titrations where the hydrogen ion concentration remains practically constant during the titration and is not a function of the titrant, *i.e.*, where the solution contains excess of acid or base or an effective buffer. Lykken and Tuemmler[7] have published results showing typical potential - volume curves for argentimetric and oxidation - reduction titrations. The titrations selected include instances in which two different titrate ions are present and the graphs show very satisfactory two-break curves. Using 0·1 *N* alcoholic silver nitrate and a titration medium of 0·1 *N* alcoholic sodium acetate, it is possible to determine hydrogen sulphide and thiophenol quantitatively in presence of each other. This procedure may be compared with the Institute of Petroleum method investigated by Davies and Armstrong for estimating mercaptans in petroleum products.[8] In the latter instance, hydrogen sulphide is first removed by shaking with cadmium sulphate and then the titration is carried out by means of a silver - alcoholic sodium acetate - mercury electrode system. Elementary sulphur is determined at the same time, giving an early break in the titration curve due to the greater insolubility of silver sulphide as compared with silver mercaptide. Temele and Ryland,[9] who first devised this method, suggested the possibility of determining both hydrogen sulphide

* pK_a = acid strength expressed as the negative exponent of the logarithm of the acidity constant K_a.

and mercaptan when present together, but favoured the removal of any elementary sulphur by prior shaking with mercury, in view of the possible reaction between the sulphur and the mercaptan in the alkaline conditions of the titration.

Other argentimetric titrations, using the glass reference electrode, include the co-determination of iodide and chloride ions and also thiocyanate and chloride. Oxidation - reduction examples, include titration of stannous and ferrous ions, singly or together, with ceric sulphate, and titration of permanganate, vanadate and bichromate with ferrous sulphate. The advantages of using an easily prepared and non-contaminating reference electrode in all these methods are apparent.

A useful potentiometric method is that using the "bottled end-point" described by Callan and Horrobin,[10] in which the reference electrode potential should be the same as the indicator electrode potential at the end-point. An interesting application of the titration of chloride ion using the "bottled end-point" is the method described by Haslam and Sweeney[11] for the determination of aniline in presence of secondary and tertiary amines. The reaction between aniline and picryl chloride is utilised and sodium bicarbonate is present to neutralise and fix the hydrochloric acid liberated in the reaction.

A noteworthy recent contribution of oxidation - reduction type is the determination of nitroguanidine[12] by decomposition with concentrated sulphuric acid and titration of the nitrate ion with ferrous ammonium sulphate by means of a platinum - tungsten electrode system. The method is of interest because of its possible application to the determination of nitro-urea.

Polarisation end-points provide a type of titration which is finding increasing use. A characteristic feature is the rapidity with which the system reaches an equilibrium at each addition of titrant near to the end-point as opposed to the pause usually necessary with orthodox potentiometric end-points. Foulk and Bawden[13] described the application of the "dead-stop end-point" to iodine - thiosulphate titrations. They used a small e.m.f. of the order of 15 millivolts between two platinum electrodes in the titrate solution, this e.m.f. being ultimately balanced by the back e.m.f. of polarisation of one of the electrodes. They deduced that a dead-stop end-point would occur in all reactions which coincided with the sharp transition from the polarisation of at least one electrode to the complete depolarisation of both of them or *vice versa.*

The application of this end-point to the determination of dissolved oxygen in water and its advantage over the starch indicator end-point have been described by Evans and Simmons[14] and Sillars and Silver.[15] Another application is the titration of ascorbic acid with 2:6-dichlorophenolindophenol in coloured solutions.[16] In this titration, excess of dye depolarises the cathode and the dead-stop end-point is observed as a permanent current surge. The response is instantaneous and has that advantage over the potentiometric titration with a mercury-coated platinum and calomel electrode system.

ELECTROMETRIC TITRATION WITH KARL FISCHER REAGENT

The importance of the Karl Fischer reagent[17] as a quantitative reagent for the determination of small amounts of water is evident from the volume of published work on this subject. It would appear that the applications are almost without limit and, in fact, foundation for further work in the quantitative analytical organic field has been truly established by the work of Smith, Bryant and Mitchell.[18] They have utilised the production or use of water in a number of organic reactions as a means of estimating organic radicals. Alcoholic hydroxyl groups may be acetylated and methyl esters formed from aliphatic acids, both with the production of a stoichiometric amount of water. Similarly carbonyl compounds can be determined from the water produced by their reaction with hydroxylamine hydrochloride. Acid anhydrides are hydrolysed with a slight excess of water, with sodium iodide as a catalyst, and the unused water determined. Other examples of the application of Karl Fischer reagent are the determination of water of hydration of salts, analysis of mixtures of primary, secondary and tertiary amines, and the determination of nitriles and amino alcohols.

Karl Fischer had suggested the use of an electrometric end-point, where the visual titration was not possible, and later Almy, Griffin and Wilcox[19] described the potentiometric back titration of excess of Fischer reagent with a standard water solution, using a platinum - tungsten electrode pair. Disadvantages were the usual wait until equilibrium was reached after each addition and the need for resensitising the tungsten electrode. The application of the "dead-stop end-point" of Foulk and Bawden was suggested by Wernimont and

Hopkinson,[20] whose method involved less manipulative difficulties. Further refinements were introduced by McKinney and Hall,[21] who used a "magic eye" indicator and a titrimeter circuit similar to that of Serfass. In both of these "dead-stop end-point" methods a potential of the order of 15 millivolts is impressed on the two platinum electrodes. Polarisation of the cathode occurs when the iodine is totally consumed at the end-point, the resulting back e.m.f. counterbalancing the applied e.m.f. to give no observable current. It was stated that the direct titration of water-containing solutions with Fischer reagent gave premature and fading end-points. Later workers[22,23] showed that a direct titration could be carried out satisfactorily. A much higher e.m.f. of the order of 1 to 2 volts can be applied and a sensitive galvanometer used by shunting with a small resistance.

It is the accepted theory that in the direct titration the cathode is depolarised by excess of iodine at the end-point. The writer has made measurements of change of resistance and current and finds that support for this theory can be obtained by calculating both the apparent electrode potential and the applied potential across the electrodes throughout the titration. The graphical representation of the change of current and resistance of the electrode system as the titration proceeds is shown in Fig. 1. The resistance was measured at each addition, using an ohm meter. The circuit was as described by Carter and Williamson,[22] and consists of a 2-volt cell, the electrode pair, a 5000-ohm resistance and the galvanometer in series, the latter having a 10-ohm shunt resistance. The galvanometer used in this experiment had a resistance of 808 ohms and a sensitivity of 60 mm. per micro-amp. It will be seen from the circuit that, if R_e is the resistance of the electrodes immersed in the titrated solution, then at any point in the titration the applied potential drop across the electrodes may be calculated from the resistance, by using the expression

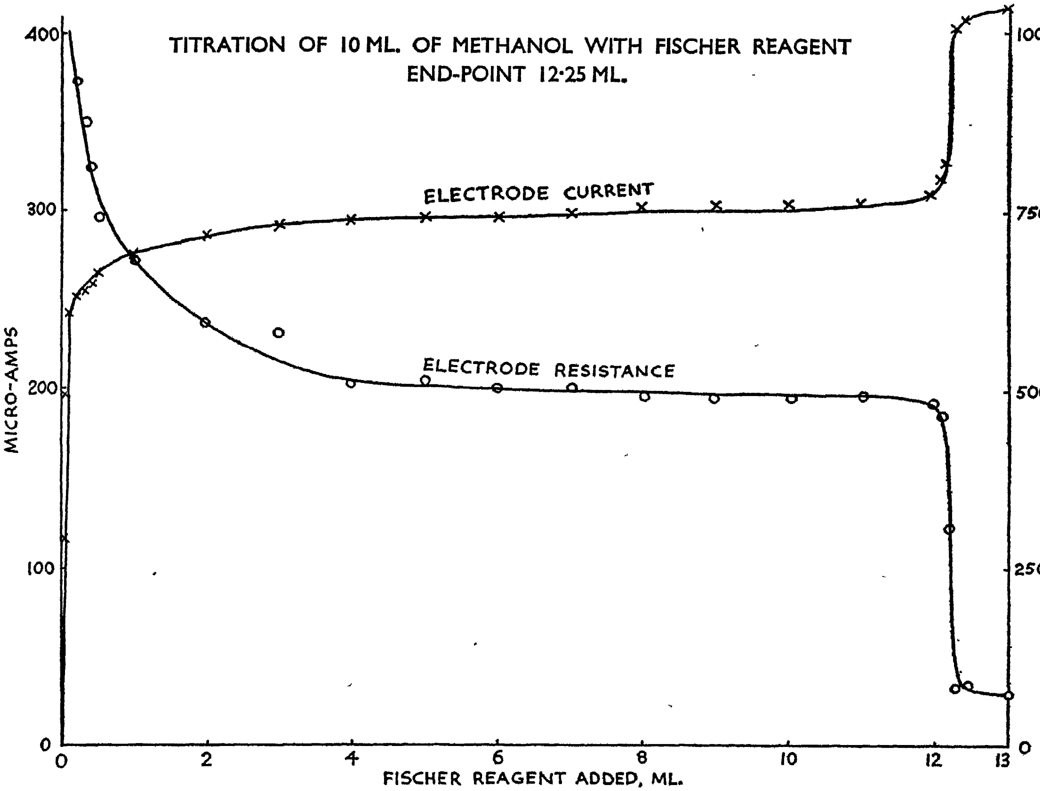

FIG. 1

$$\text{Applied potential} = \frac{R_e}{R_e + 5010} \times 2 \text{ volts.}$$

The apparent potential may be deduced from the current, by using the expression

$$\text{Apparent potential} = I_e \times R_e \times 10^{-6} \text{ volts},$$

where I_e is the current flowing across the electrodes, in micro-amps., *viz.*, galvo current × (galvo + shunt resistance) ÷ shunt resistance. It was found that at the commencement of the titration the e.m.f. applied was approx. 0·3 volt greater than the apparent e.m.f., and that this difference decreased rapidly to a value of about 30 millivolts, which was maintained during the greater part of the titration. We may regard this difference as a measure of the back e.m.f. or polarisation e.m.f. At the end-point of the titration the value suddenly decreases to zero, corresponding to the depolarisation of the cathode. The values derived are shown graphically in Fig. 2.

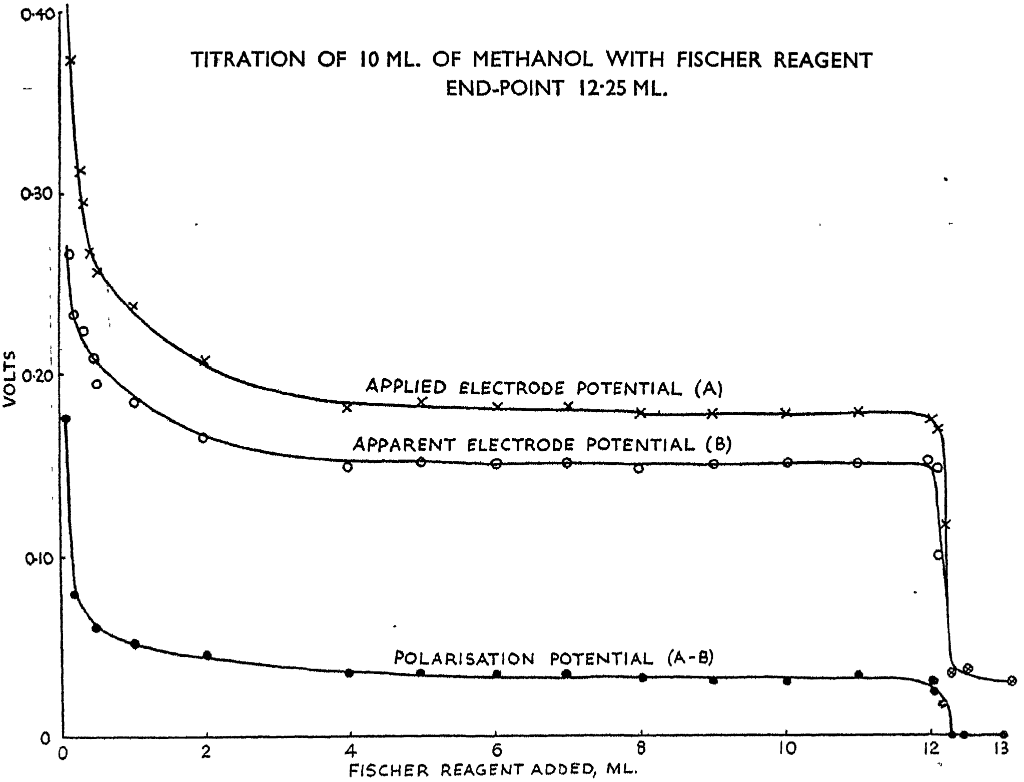

FIG. 2

CONCLUSION

It is suggested that electrometric methods of most types are bound to become a routine procedure in the future and not remain isolated as a special technique by a special operator. Simplification of apparatus and compactness of set-up will help greatly towards making the methods more suitable for general use, and in this respect it is pleasing to note that compact commercial titrimeters are now becoming available. It may be that bi-metallic electrode methods and polarisation end-point methods will find increasing application where possible. In view of the high degree of accuracy often obtainable with electrometric methods it is essential that these methods, where practicable, be utilised as readily as other standard analytical procedures and not be regarded merely as an extraordinary measure when other alternatives fail.

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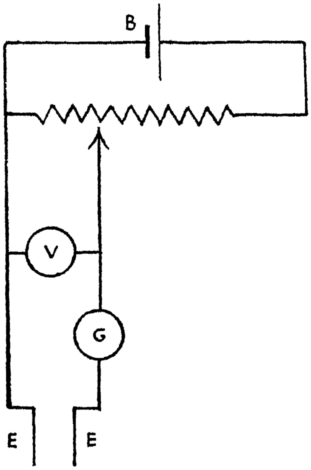
Polarisation End-points

BY D. P. EVANS

IN the three main methods of determining end-points in volumetric analysis, use is generally made of either (1) indicators, (2) an indicator electrode together with a reference electrode, or (3) polarised electrodes. When indicators are employed we usually obtain a rapid and accurate titration in clear colourless liquids but difficulties occur with coloured and turbid liquids, and for routine work in artificial light the possibility of eye-strain cannot be overlooked. In the more usual electro-titrations a salt bridge is necessary between the solution containing the indicator electrode and the standard half-cell. This objection (*i.e.*, the use of a salt bridge) may, however, be obviated by use of a glass electrode as the standard, provided that the *p*H does not alter appreciably during titration. But both methods involve the use of fairly elaborate and expensive apparatus and it is necessary to wait for equilibrium to be reached before accurate readings may be obtained.



E E denote the two platinum wire or plate electrodes immersed in the test solution, which is provided with a mechanical stirrer.
G is a reflecting galvanometer (sensitivity 22 cm. per micro-amp.).
V is a millivoltmeter.
B represents a small dry battery.

Fig. 1.

The third method depends on the polarisation or depolarisation of one or two inert electrodes at the end-point. The idea was investigated in some detail by van Name and Fenwick,[1] and later by Foulk and Bawden.[2] The latter authors suggested a very simple and inexpensive apparatus which gives surprisingly accurate results under the correct conditions. The other advantages of the method consist in the very rapid—practically instantaneous—development of equilibrium, and the possibility of its use in turbid and coloured solutions.

When an external potential is applied to two platinum electrodes immersed in an acid solution, a back e.m.f. is developed due to deposition of a thin layer of hydrogen on the cathode and one of oxygen on the anode. If the applied e.m.f. is appreciably less than the maximum back e.m.f. of the system, the current in the circuit soon falls to zero. Usually in the polarisation end-point method an e.m.f. of about 10 to 20 millivolts is applied and the galvanometer registers zero current instantaneously. The circuit is illustrated in Fig. 1 on p. 99.

In order to illustrate the method its application to the titration of thiosulphate with iodine will be described. When the electrodes are immersed in a solution of thiosulphate and the applied e.m.f. is switched on, the galvanometer immediately registers zero (or almost zero) current because the back e.m.f. immediately becomes equal to the applied e.m.f. Owing to the reducing action of thiosulphate anions, however, the oxygen is completely removed from the anode and the whole of the back e.m.f. is due to polarisation of the cathode. On addition of a little iodine, the galvanometer spot makes a temporary excursion from the zero position, owing to partial depolarisation of the cathode. The hydrogen thus removed is rapidly replaced and the spot returns to zero. At the end-point, however, the presence of the slightest excess of iodine causes a permanent small depolarisation of the cathode and the applied e.m.f. is then greater than the maximum back e.m.f. which may be developed and the galvanometer registers a small deflection due to current moving in the appropriate direction. Further addition of iodine results in a larger deflection of the galvanometer spot.

If the thiosulphate is added to the iodine there is at first a current which becomes zero at the end-point, hence the term "Dead-Stop End-Point." For accuracy the former method is better.

There is one essential condition for success in the above titration, *viz.*, the anode must be completely depolarised throughout the titration. Two substances can depolarise the anode

(i) Thiosulphate anions:

$$2S_2O_3'' + H_2O + O \longrightarrow S_4O_6'' + 2OH'.$$

(ii) Iodide ions:

$$2I' + H_2O + O \longrightarrow I_2 + 2OH'.$$

In 0·1 N solutions of iodine there is sufficient potassium iodide to ensure the above condition even at the end-point, but with 0·002 N solution, as in the determination of dissolved oxygen in boiler-feed water, as suggested by Hewson and Rees,[3] this is not so. Near the end-point, in the absence of sufficient iodide ions, the accidental current which passes on addition of each drop of iodine to the thiosulphate solution causes polarisation of the anode, with the result that the "back e.m.f." may rise slightly above the applied e.m.f. An amount of iodine somewhat greater than the equivalent must then be added to depolarise the cathode sufficiently to give a current in the direction required by the end-point condition: applied e.m.f. > back e.m.f.

The following table shows the effect of added 10 per cent. potassium iodide solution on the titre of 0·002 N iodine dissolved in 0·04 per cent. iodide solution required by 1 ml. of 0·002 N thiosulphate in 250 ml. of water.

Experiment No.	Potassium iodide solution added ml.	Iodine titre ml.
1	0·0	1·16
2	0·5	1·08
3	1	1·06
4	2	1·07
5	4	1·01
6	5	1·01
7	10	1·00

When 5 ml. of potassium iodide solution are added the end-point is given accurately even at the dilution employed (1 ml. of 0·002 N in 250 ml. of water). Under these conditions starch is totally inadequate as an indicator, yet the polarisation end-point affords an accuracy of ±0·01 ml. of 0·002 N I_2 or ±0·0005 ml. of oxygen per litre—an essential requirement when the oxygen content of the boiler feed water must be less than 0·02 ml. per litre (*cf.* Evans and Simmons[4]).

Other applications consist in the measurement of chlorine in domestic water supplies, water in oils, and other oxidation - reduction reactions.

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MINING AND TECHNICAL COLLEGE
BRIDGEND, GLAMORGAN

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DISCUSSION

following the reading of the preceding three papers

MR. C. H. R. GENTRY remarked that although the Cambridge *p*H meter will enable readings to be made to 0·01 *p*H unit, he doubted if it was justifiable to report *p*H measurements to this degree of precision. Co-operative studies made in America appeared to indicate that the accuracy of *p*H measurements with the glass electrode is nearer 0·05 unit. Those studies were made on buffer solutions, and as in practice measurements are often made on poorly buffered, poorly poised solutions a direct-reading *p*H meter measuring to 0·1 unit would probably suffice in industrial analyses. The speaker had found that the "magic eye" in commercial potentiometric apparatus had much to recommend it; with a little care e.m.f.'s could be read with it to within 2 millivolts—a value comparable with that given by galvanometer-type instruments.

MR. LAUCHLAN pointed out that 2 millivolts correspond to nearly 0·05 *p*H, hence his preference for the galvanometer.

MR. J. JACKSON said Mr. Gentry might be interested in a semi-statistical examination of the accuracy of commercial glass electrode *p*H meters carried out in America, and reported in *J. Assoc. Off. Agric. Chem.*, 1942, **25**, 973–980 (see also *Ibid.*, 1945, **28**, 579). The method was to send out from a central laboratory bottles of solids, the contents of which were dissolved in distilled water at the receiving laboratory, and the *p*H of the solution was measured, a prescribed solution being used to standardise the *p*H meter. The standard deviation of the accepted results was 0·02 to 0·03 *p*H units. The speaker agreed with Mr. Lauchlan, however, that with careful use the reproducibility of the e.m.f. of a glass electrode cell is considerably better than this figure suggests.

Mr. Carter had referred to the increasing use of *p*H measurements in non-aqueous media. When an ordinary reference electrode is used difficulties are occasionally encountered owing to precipitation at the liquid junction. For example, this occurs with solutions of synthetic resins in mixtures of toluene and ethyl alcohol. It is best then to use a reversible electrode and salt bridge solution, employing an appropriate solvent. Frumkin, in connection with electro-capillary measurements, has described reversible electrodes in a number of alcoholic and other media (*Z. phys. Chem.*, 1922, **103**, 43).

The speaker expressed interest in the chlorine-in-water meter described by Dr. Evans. Presumably what is measured by it is the diffusion current of chlorine to the noble metal electrode, maintained at a constant potential. This current, however, is very susceptible to impurities adsorbed on and in the neighbourhood of the electrode surface. Wallace and Tiernan have patented one or two quite intricate devices to maintain a clear electrode surface in such an instrument. He would like to ask Dr. Evans if the one he described does hold its calibration.

DR. EVANS replied that it does.

Spot-tests for the Detection of Alloying Elements in Zinc-Base Alloys*

BY B. S. EVANS AND D. G. HIGGS

THE mechanical properties of zinc-base alloys are such that only a few have been found suitable for general purposes. The usual elements alloyed with zinc are aluminium, copper and lead. Aluminium solders of the zinc-base type have occasionally been employed in industry; these, unlike the constructional alloys, have a variety of alloying elements. Sometimes binary alloys are used for the purpose of soldering but there have appeared alloys of quite complex compositions. The tests to be described in this paper have been designed to be applicable to all the commercial zinc-base alloys.

As far as possible, tests described in previous papers[1,2] have been used, with suitable modifications where necessary and with no needless departure from the usual technique. Some new techniques and reagents, however, have become necessary.

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* Communication from the Armament Research Department; formerly the Research Department, Woolwich.

Very few samples have been available for the evolution of these tests; the majority have been made and analysed by one of the authors. Whilst every effort has been made to make the tests universally applicable, no guarantee can be given that they will work with alloys of a complex character that have not been encountered in the course of this investigation.

Apparatus—The requirements for these tests are (*a*) short lengths of narrow glass tubing drawn out into long fine jets, (*b*) stirring rods similarly drawn out into points, (*c*) a few small watch-glasses of about two inches diameter, (*d*) 400-ml. squat beakers on which to support the papers of tests (II) and (VI), (*e*) a small dish 2 inches in diameter and 1·5 inches deep, (*f*), white porcelain tile and (*g*) Petri dishes of about 6 inches diameter.

THE TESTS—The samples must be cleaned by rubbing with an emery paper of fairly coarse texture before the tests are applied.

In all but one of the following tests the reaction products have to be removed from the surface of the sample for completion of a test; this is due to the highly reactive nature of the base metal. In two instances, those of tin and lead, addition of aluminium in the form of small chips or coarse powder to the surface before adding the attacking reagent has been found necessary. This innovation facilitated solution of the tin from the alloy and, in the case of lead, left on the metal a loose deposit which could be easily separated from the base metal for subsequent treatment.

Compositions of trial alloys are given in the Appendix (page 105), and the numbers at the end of each test refer to that Appendix.

(I) COPPER

Reagents—(*a*) Diluted nitric acid (sp.gr. 1·20).
(*b*) Mixture of α-benzoin-monoxime in alcohol (saturated solution), 10 vols.; diluted ammonia (1+1), 20 vols.; citric acid solution (50 per cent.), 5 vols.
(*c*) Hydrogen peroxide (20 vol. solution).

Method—Add 1 drop of (*a*) to the thoroughly cleaned surface and leave it to react for 1 to 2 minutes. Then add 6 drops of (*b*), stir well, follow with 1 drop of (*c*) and stir again. In presence of copper a dirty green precipitate quickly develops. In absence of copper there may be a slight white precipitate due to the reagent.

Tried on: Samples Nos. 8, 1, 9—Copper present; all gave positive results.
Nos. 2, 4, 5, 6, 7—Copper less than 0·10 per cent. and all results negative.

(II) ALUMINIUM

Reagents—(*a*) Hydrochloric acid (5 per cent. solution) saturated with bromine.
(*b*) Mixture of sodium hydroxide solution (20 per cent.), 1 vol.; potassium cyanide solution (10 per cent.), 1 vol.
(*c*) Ammonium chloride solution (20 per cent.).
(*d*) Ammonium aurintricarboxylate solution (0·1 per cent.) in alcohol.

Method—Prepare a disc of fairly close-grained filter-paper by running on to it 1 ml. of (*d*), causing the liquid to spread as evenly as possible and allowing to dry; lay the prepared paper on the open mouth of a beaker. Place on the thoroughly cleaned sample 1 drop of (*a*) and allow it to become decolorised. Add 4 or 5 drops of mixture (*b*) and stir thoroughly. Transfer the liquid, by means of a capillary tube, to the centre of the paper in a dropwise manner. When the last drop has been completely absorbed lay the paper flat on a clean tile and cover it with a piece of filter paper which has been soaked in (*c*) and allowed to drain. Allow the covering paper to remain in position for a few seconds, then strip off, return the other paper to the top of its beaker and leave for 15 minutes. Wash the paper about 6 times with 3 or 4 drops of (*c*), dropped into the centre and allowed to spread before the next wash is added, and then leave to dry.

Aluminium is indicated by a scarlet ring, about 1½ to 2 inches from the centre, sharply defined on the inner and somewhat diffused on the outer side; it is usually ⅛ to ¼ inch broad. The ground within the ring should be white, that outside generally a purplish band of the dye itself. The record on the paper seems to be quite stable and, after drying, will keep indefinitely.

Tried on: Samples Nos. 5, 9, 1, 2, 8, 3, 6—Aluminium present; all gave positive results.
Nos. 4, 7—Aluminium absent and both results negative.

(III) ANTIMONY

Reagents—(*a*) Mixture of tartaric acid solution (50 per cent.), 1 vol.; saturated bromine water, 1 vol.
(*b*) Hydrochloric acid solution (10 per cent.).
(*c*) Sodium hypophosphite (solid).
(*d*) Copper foil (approximately 3/16 inch square).

Method—Place 6 drops of (*a*) on the thoroughly cleaned surface and allow to react until the bromine colour has nearly disappeared; quickly wash the drops into a 60-ml. tall-form beaker, with a few drops of water. Add 10 ml. of (*b*) to the reaction drops and then about 0·5 g. of (*c*) and the piece of copper foil (*d*), previously cleaned with nitric acid, taking care not to touch the copper foil with the fingers. Boil the solution gently until the salts begin to crystallise and the volume of the liquid is about 0·5 to 1 ml.; then cool and dilute with water. With small quantities of antimony it is only after the salts begin to crystallise that the antimony deposits on the copper. Place the beaker on a white glazed tile for comparison with a blank test carried out under exactly the same conditions; comparison should be made by looking vertically downwards on to the beakers.

Only one sample containing antimony was available, *viz.*, No. 6; this gave a positive reaction.

Tried on: Samples Nos. 1, 2, 4, 5, 7, 8, 9—Antimony absent and all results negative.

(IV) TIN

Reagents—(*a*) Mixture of tartaric acid solution (50 per cent.), 4 vols.; diluted nitric acid (sp.gr. 1·20), 2 vols.; potassium cobalticyanide solution (10 per cent.), 1 vol.
(*b*) Mixture of diluted hydrochloric acid (15 per cent. v/v), 4 vols.; potassium cobalticyanide solution (10 per cent.), 1 vol.
(*c*) Mixture of toluene-3:4-dithiol[3] solution (0·5 per cent.) in water-free acetone (prepared fresh daily), 1 vol.; thioglycollic acid solution (0·5 per cent.) in acetone, 1 vol.
(*d*) Aluminium chips or coarse powder.

Method—To the thoroughly cleaned surface add a small quantity of (*d*) followed by 4 drops of (*a*), mix-in the aluminium and then leave to react for 5 minutes. Add 6 drops of (*b*), stir, and leave for 1 to 2 minutes. Transfer the solution to a watch-glass either by means of a capillary tube, or by tilting the sample so that the liquid runs on to the watch-glass. Add 4 drops of (*c*), stir and leave to react. In presence of tin the white precipitate that first forms turns red slowly. In absence of tin the colour of the precipitate varies from a pale yellow to a grey; the yellow is due to zinc (and cadmium if present) both of which slowly fade to a dirty white; the grey colour is due to copper, not precipitated by the cobalticyanide, giving a black precipitate with the reagent.

In presence of large amounts of copper the greater part of the tin present is co-deposited as the copper-tin compound[4]; sufficient remains in solution, however, to give a strong reaction. If, after the bulk of the liquid has been removed from the sample as described above, the remaining liquid is allowed to dry and the crust removed by scraping with a knife or razor blade, a considerable reaction for tin is given when the crust is treated with the reagents as described in the test.

Tried on: Samples Nos. 6, 8, 9—Tin present; all gave positive results.
Nos. 7, 4, 5, 1, 2—Tin absent and all results negative.

(V) CADMIUM

Reagents—(*a*) Mixture of diluted nitric acid (sp.gr. 1·20), 1 vol.; citric acid solution (50 per cent.), 1 vol.
(*b*) Mixture of diluted ammonia (1+1), 1 vol.; ammonium nitrate solution (20 per cent.), 1 vol.
(*c*) Mixture of potassium cyanide solution (20 per cent.), 3 vols.; sodium sulphide solution (10 per cent.), 1 vol.

Method—To the thoroughly cleaned surface add 2 drops of (*a*) and leave until the vigorous reaction has ceased; wash the drop off the sample, by means of a fine jet of distilled water, on to a watch-glass. Add 4 drops of (*b*), stir, then add 6 drops of (*c*), stir again and leave for the cadmium sulphide precipitate to develop. In absence of cadmium the mixed drops remain clear. Cadmium gives a bright yellow precipitate. A series of zinc - cadmium alloys were prepared containing amounts of cadmium from 20 per cent. down to 1 per cent.; all gave visible yellow precipitates, although those from the 2·5 per cent. and 1·0 per cent. solutions were rather slow in forming.

Tried on: Samples Nos. 7, 10, 11, 12, 13, 14—Cadmium present; all gave positive results.
Nos. 8, 9, 4, 5, 6, 1, 2—Cadmium absent and all results negative.

(VI) LEAD

Reagents—(*a*) Diluted nitric acid solution (sp.gr. 1·20).
(*b*) Concentrated hydrochloric acid saturated with bromine.
(*c*) Diluted ammonia (1+1).
(*d*) Mixture of ammonium acetate solution, 2 vols.; sodium chromate solution (5 per cent.), 1 vol. To prepare the ammonium acetate solution, add to 1140 ml. of distilled water in a 3-litre porcelain beaker placed in a cooling bath 500 ml. of concentrated ammonia (sp.gr. 0·880) followed by 570 ml. of glacial acetic acid, with continuous stirring, and allow to cool; the solution should be neutral.
(*e*) Mixture of potassium cyanide solution (5 per cent.), 9 vols.; dithizone solution (0·1 per cent.) in chloroform, 1 vol.
(*f*) Aluminium chips or coarse powder.
(*g*) Acetone.

Method—Place a small quantity of (*f*) on the thoroughly cleaned surface, add 4 drops of (*a*), stir-in the aluminium and leave to react for 5 minutes. Transfer the drops, by means of a capillary tube, to a watch-glass. Remove the aluminium from the surface of the sample by washing with acetone and then leave to dry. Carefully remove the deposit from the surface of the sample, by means of a sharp knife or razor blade and drop it on to the liquid already on the watch-glass. Add 3 drops of (*b*) and stir with a pointed glass rod, until all is dissolved. Place a 9-cm. No. 541 Whatman filter paper on the open mouth of a beaker, transfer the solution from the watch-glass on to the centre of the paper and allow it to spread. Place the paper on top of a dish, of about 2 inches diameter, containing (*c*) and leave, covered, for 1 minute, by which time all the free acid should be neutralised. Transfer the paper to a Petri dish containing (*d*), allow it to soak for a few seconds and then remove and drain it for 30 seconds. Next wash the paper for 2 to 3 minutes in running water, transfer it to a second Petri dish containing (*e*) and develop, with occasional agitation, for 15 minutes; again wash in running water until the colour of the paper has changed from golden brown to a light pink and hang it up to dry. Lead is indicated by a red patch covering the whole of the original wet area; sometimes there is a tendency for the lead to accumulate, forming a ring of denser colour at the outer edge. The rest of the paper should be white, or a very pale shell-pink, when dry. In absence of lead the paper is white or a uniform pale shell-pink.

Reagent mixture (*e*) should be thoroughly mixed before use, the cyanide having a golden colour; when the chloroform layer begins to appear brownish, instead of its usual black colour when freshly prepared, it is an indication that the mixture (*e*) should be renewed.

Tried on: Samples Nos. 6, 7, 8, 9—Lead present; all results positive.
Nos. 1, 2, 4, 5, 10, 11, 12—Lead absent and all gave negative results.

NOTE—The presence of lead in sample No. 7 was discovered by this test; subsequent analysis showed that the sample contained 0·26 per cent., and that the cause of its introduction into the alloy was the supposedly pure cadmium. Alloys Nos. 10–14 were prepared from pure zinc and cadmium.

SUMMARY

Tests are described for the detection of alloying elements in zinc-base alloys. The alloying elements so detected are copper, aluminium, antimony, tin, cadmium and lead.

With the alloys at our disposal the tests were found to be specific and unambiguous.

Thanks are due to the Chief Scientist, Ministry of Supply, for permission to publish this paper.

APPENDIX

SPECIMENS OF ALLOYS USED IN THE TESTS. CONSTITUENTS PER CENT.

No.	Mark	Al	Cu	Pb	Mg	Fe	Sb	Sn	Cd	Remarks
1	210	4·30	1·01	—	0·054	0·01	—	—	—	
2	209	4·14	0·03	—	0·052	0·008	—	—	—	
3	L/AUW	4·05	0·003	0·004	0·05	—	—	—	—	
4	Zinc	—	—	—	—	—	—	—	—	
5	JQW	21·5	—	—	—	—	—	—	—	Made up and analysed by one of the authors. No. 6 is of nominal composition only.
6	DKL(N)	0·25	0·05	1·5	—	—	0·15	18·5	—	
7	EX.8	—	—	0·26	—	—	—	—	44·0	
8	EX.10	3·80	2·18	0·74	—	—	—	24·0	—	
9	EX.9	15·0	0·84	0·43	—	—	—	6·53	—	
10	EX.69	—	—	—	—	—	—	—	20	Nominal composition only; not actually analysed.
11	EX.70	—	—	—	—	—	—	—	10	
12	EX.71	—	—	—	—	—	—	—	5	
13	EX.72	—	—	—	—	—	—	—	2·5	
14	EX.73	—	—	—	—	—	—	—	1·0	

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January, 1947

Spot-tests for the Detection of Alloying Elements in Lead-base Alloys*

BY B. S. EVANS AND D. G. HIGGS

LEAD-BASE alloys have found extensive application in industry, with the result that there is a wide range of alloying elements. Specifications[1] for these alloys include the elements tin, antimony, cadmium, silver, arsenic, bismuth and tellurium in various percentages and admixtures. The samples used in the course of developing these tests have been made up either from the above-mentioned specifications, or from alloys previously analysed in this laboratory; all have been analysed by one of the authors.

Tests are described for the following elements: tin, antimony, cadmium, silver, arsenic and bismuth. Except those for bismuth and cadmium, which are modifications of tests described in an earlier paper,[2] the tests described are entirely new to our programme of spot-testing. The antimony test is a departure from the Reinsch test, to which we have strictly adhered in former papers.[2,3,4] The Reinsch test, although very reliable and delicate, is not well suited to the detection of antimony in lead-base alloys. Clarke's[5] modification of the Reinsch test permits the presence of large amounts of tin but, as difficulty might be encountered from arsenic and high bismuth contents, the method has not been tested. The test described for the detection of arsenic is a modification of Gutzeit's test. Numerous tests tried for the detection of tellurium were all unsuccessful; it is our opinion that the amount specified under S.T.A.7 or B.S.334, Type B (0·06 to 0·07 per cent. of copper; 0·04 to 0·05 per cent. of tellurium) is below the limits of detection.

Apparatus—In addition to the droppers, stirrers and capillary tubes, already described in former papers, the following are needed: (*a*) white porcelain spot-plate, (*b*) tile or glass plate covered with black paper (used in Test IV, Silver), (*c*) apparatus used for the arsenic test (*vide* Test V) and (*d*) metal scraper for cleaning the sample.

Owing to the surface plastic flow of lead when treated with light pressures, the use of emery paper for polishing is very unsatisfactory; resort has to be made to the use of a scraper. The instrument employed throughout these tests was made from a wooden-handled steel spatula. The steel blade was cut off square about 2¼ inches from the hilt. The end of the blade was made red-hot and, while still hot, bent at right angles about ¼ inch from the end of the blade. When cool, the surface of the edge facing the handle was ground to form a sharp cutting-edge. The blade was first cleaned with emery paper to present a bright surface and then heated over a small flame until the whole of the part at right angles to the handle became of a straw-yellow colour and the last ⅛ of an inch varying from light blue to deep blue at the cutting edge; it was then allowed to cool. By pressing the blade on the specimen and drawing it backwards a clean, smooth surface was obtained.

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* Communication from the Armament Research Department, formerly the Research Department, Woolwich.

THE TESTS—Of the tests described only two can be carried out completely on the surface of the sample—they are those for tin and bismuth; the tests for antimony, cadmium, silver and arsenic require removal of the reaction drops at some intermediate stage for completion.

(I) TIN

Reagents—(*a*) Mixture of diluted nitric acid (sp. gr. 1·20), 1 vol.; tartaric acid solution (50 per cent.), 1 vol.
(*b*) Diluted hydrochloric acid (15 per cent. v/v).
(*c*) Mixture of toluene-3:4:-dithiol[6] solution (0·5 per cent.) in water-free acetone, prepared fresh daily, 1 vol.; thiogylcollic acid solution (0·5 per cent.) in acetone, 1 vol.

Method—To the thoroughly cleaned surface add 2 drops of (*a*) and leave to react for 5–10 minutes; then add 4–5 drops of (*b*) followed by 3–4 drops of (*c*), stir, and leave to react. In presence of large amounts of tin an immediate red precipitate is observed, with about 5 per cent. of tin the development of the precipitate is somewhat delayed and, for 0·5 per cent. of tin at least 5–10 minutes may be required before the colour develops. Bismuth does not give its reddish-brown precipitate under the conditions of the test. In absence of tin all other alloys give either varying shades of yellow or no precipitate at all. The reactions are somewhat less intense when silver is present in the alloy, *e.g.*, there does not appear to be much difference in intensity between the reactions for 5 per cent. and 50 per cent. of tin.

Tried on: Samples Nos. 18, 17, 19, 10, 20, 15, 21, 7, 23, 24, 25, 26, 27, 28, 29, 30—Tin present; all results positive.
Nos. 22, 31, 2, 4, 6, 8, 9, 13, 14, 16, 36—Tin absent and all results negative.

(II) ANTIMONY

Reagents—(*a*) Concentrated hydrochloric acid saturated with bromine.
(*b*) Concentrated hydrochloric acid.
(*c*) Potassium nitrite (solid).
(*d*) Rhodamine "B"[7] solution (0·01 per cent.) in water.

Method—Add to the thoroughly cleaned surface 3 drops of (*a*) and allow to react until the bromine colour has just become dispelled. Withdraw the drop, by means of a capillary tube, together with as much of the white oxide precipitate as possible (this precipitate results from high antimony contents) and place it in a well of a white porcelain spot-plate. Add 2 drops of (*b*), stir and follow with a few small crystals of (*c*), stir again and then leave for about 1 minute. Add 4 drops of (*d*) to another well of the spot-plate, transfer 2 drops from the first well to the reagent and stir thoroughly. In presence of antimony in proportions down to about 1·5 per cent. an immediate purple coloration is observed. Antimony contents of about 0·5 per cent. give a purple-mauve reaction which fades rapidly if the tin content of the sample is high. In presence of silver, all antimony contents give the reaction, but all are subject to somewhat rapid fading. With lead - antimony alloys a strong purple-mauve reaction is given by 0·25 per cent. of antimony. In absence of antimony the colour of the drop varies from a dirty brick-red to a reddish pink.

Tried on: Samples Nos. 1, 2, 6, 7, 8,9, 11, 13, 15, 16, 17, 18, 19, 20, 21, 25, 26, 28, 29, 33, 34, 32—Antimony present; all gave positive results.
Nos. 4, 10, 14, 22, 24, 27, 30, 31, 36—Antimony absent and all results negative.

(III) CADMIUM

Reagents—(*a*) Diluted nitric acid (sp.gr. 1·20).
(*b*) Potassium cobalticyanide solution (10 per cent.).
(*c*) Mixture of cobalticyanide solution (10 per cent.), 1 vol.; nitric acid solution (sp.gr. 1·20), 1 vol.

(*d*) Mixture of diphenylcarbazone solution (1·5 per cent.) in alcohol, 1 vol.; saturated borax solution, 1 vol.*
(*e*) Half-saturated borax solution.
(*f*) Ethyl alcohol.

Method—Attack the cleaned surface with 2 drops of (*a*) and allow to react for 3–4 minutes; then add 2 drops of (*b*), stir, and leave for 1 minute. Remove the drops, by means of a capillary tube, and drop them on to the centre of a fine-grain filter-paper, supported on the open mouth of a beaker, allowing each drop to become completely absorbed before adding the next. When all the transferred liquid has been added to the paper and the last drop has completely soaked in, add 2 drops of (*c*); when this has disappeared follow with two 2-drop washings of (*b*), again allowing each wash to disappear before adding the next. Add 3 drops of (*d*) and leave to spread; it is important for the following reactions that the last drop of (*b*) should have completely disappeared before addition of (*d*). Continue by washing the reagent patch with two 4-drop washings of (*e*). Finally add to the centre of the reagent patch, in a slow dropwise manner, 4 drops of (*f*); allow to finish spreading, then add a further two 4-drop washings and leave until dry. When dry the paper will show, if cadmium is present, a dark purple irregular ring, about 1½ to 2 inches in diameter, just inside the outer mauve edge of the reagent patch. With cadmium contents of, say, 0·18 per cent., this dark purple ring may not be more than a line. In absence of cadmium the outer edge of the reagent patch forms a mauvish-purple band, about ¼ inch wide, but no sign of the dark purple irregular ring appears.

Tried on: Samples Nos. 3, 4, 5, 6, 7, 11, 12—Cadmium present: all gave positive results.
Nos. 2, 8, 13, 14, 15, 16, 18, 22, 36—Cadmium absent and all results negative.

(IV) SILVER

Reagents—(*a*) Diluted nitric acid (sp.gr. 1·20).
(*b*) Potassium cyanide solution (20 per cent.).
(*c*) Ammonium acetate solution, 4 vols.; potassium iodide solution (4 per cent.), 1 vol.; acetic acid (glacial), 1 vol. To prepare the ammonium acetate solution, add to 1140 ml. of distilled water in a 3-litre porcelain beaker placed in a cooling bath 500 ml. of concentrated ammonia (sp.gr. 0·880) followed by 570 ml. of glacial acetic acid, with continuous stirring, and allow to cool; the solution should be neutral.

Method—To the thoroughly cleaned surface add 2 drops of (*a*) and allow to react for 5 minutes. Remove the excess of acid by touching the edge of the drop with the end of a capillary tube. To the wet patch resulting from the previous operation add 3 drops of (*b*), stir thoroughly and then leave for 5 minutes. Transfer the cyanide drop from the sample to a small watch-glass; this is best done by placing a capillary tube at the edge of the drop and tilting the sample slightly, the object being to effect removal of the liquid (together with some lead cyanide) without disturbing any of the black metallic deposit which adheres loosely to the sample. Place the watch-glass, containing the transferred cyanide drops, on a black background (*vide* Apparatus (*b*), page 105). Add 6–8 drops of (*c*), stir well until all the lead cyanide has dissolved, then leave for 15 minutes. In presence of silver an immediate pale yellow precipitate or whitish yellow haze is produced; in both instances the silver iodide may be accompanied by metallic silver, giving the drop a dark appearance. The halide precipitate, or haze, is clearly seen against the black background. In absence of silver all samples give a *clear*, colourless solution.

Tried on: Samples Nos. 22, 23, 24, 25, 26, 27, 28, 29, 30—Silver present; all gave positive results.
Nos. 2, 4, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 31, 36—Silver absent and all results negative.

(V) ARSENIC

Reagents—(*a*) Diluted hydrochloric acid (1+1) saturated with bromine.
(*b*) Concentrated hydrochloric acid.
(*c*) Arsenic-free zinc.
(*d*) Mercuric chloride test paper.
(*e*) Ferrous sulphate (small crystals).

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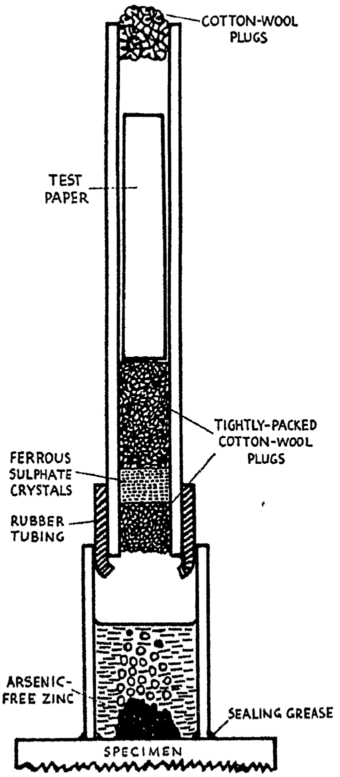
* The addition of the borax solution to the alcoholic reagent results in deposition of some of the borax; it is advisable to decant the supernatant liquid for use in the test.

Apparatus—See Figure. It comprises (*g*) a glass tube 1 inch long by ⅝ inch in diameter, ground flat at both ends, (*h*) a narrower glass tube 2¾ inches long by ⅜ inch in diameter, fitted at one end with a half-inch length of rubber tubing, a little of which overlaps the end of the glass tube (*h*), forming a slightly tapered end that serves as a stopper fitting snugly into tube (*g*), (*j*) rubberised vaseline such as is used for stop-cock lubricants and (*k*) cotton-wool.



To prepare the tube (*h*) for the test, insert a tightly packed plug of cotton-wool into the tube so that its lower end is about 0·5 inch from the bottom of the tube, the plug being about 0·5 inch deep. To the rubber-tubing end of the cotton-wool add fine crystals of (*e*) until a layer about ¼ inch thick has been formed; then insert a second cotton-wool plug to prevent the ferrous sulphate from falling out when the tube is in position for the test. Cut a strip of paper (*d*) about ¼ inch by 1¼ inch, insert it in the glass tube and then plug the free end of the tube loosely with cotton-wool; the tube is now ready for the test. (See Figure.)

Method—Scrape the specimen until the surface is both clean and flat. Dip the end of the glass tube (*g*) into the hot sealing grease (*j*), then withdraw it and, after making sure that there is no bubble film of grease across the end of the tube, press it firmly on to the sample. Add 4 drops of (*a*) inside the wide tube (*g*) and leave to react for 5 minutes. Add 15 drops of (*b*) inside the wide tube. Drop a small granule of (*c*) into the wide tube and allow the reaction to proceed for a few seconds; then, before the bromine has been destroyed, insert the rubber-stoppered end of the prepared tube (*h*) and leave for 15 minutes. Detach the top tube (*h*), remove the top plug and shake out the test strip. If arsenic is present there will be a yellow, orange or brown coloration of the middle part of the strip. If the alloy contains notable amounts of antimony, the whole of the strip will have been covered with a brown or black coloration shortly after all the bromine has been destroyed. The coloration immediately begins to fade and at the end of the 15 minutes only the lower end of the strip remains coloured. On exposure to air and light for 1 hour the antimony stain is reduced to a light grey stain or coloration at the base of the strip, whilst the arsenic stain will be plainly visible on the middle portion of the strip. Antimony contents of 2 to 3 per cent. give a slight yellow coloration at the base of the strip but this fades completely after a short exposure to the air. All other samples, not containing arsenic or high antimony, showed no coloration of the paper throughout the test.

Tried on: Samples Nos. 1, 2—Arsenic present: both gave positive results.
Nos. 8, 13, 15, 20, 25, 16, 9, 4, 6, 7, 10, 12, 14, 18, 22, 24, 28, 30, 31, 36—Arsenic absent and all results negative.
Nos. 8, 13, 15, 20, 25, 16, 9—All contain more than 7·0 per cent. of antimony and all gave the reaction described in the test above; it could in no case be confused, however, with the reaction given by arsenic.

(VI) Bismuth

Reagents—(*a*) Mixture of diluted nitric acid (sp.gr. 1·20), 1 vol.; tartaric acid solution (50 per cent.), 1 vol.
(*b*) Urea solution (10 per cent.).
(*c*) Mixture of potassium iodide solution (4 per cent.), 1 vol.; antipyrine solution (1 per cent.) in water, 2 vols.
(*d*) Sodium thiosulphate solution (2·5 per cent.).

Method—Add 2 drops of (*a*) to the thoroughly cleaned surface and leave to react for 2 to 3 minutes. Add 4 drops of (*b*), stir, then follow with 3 drops of (*c*) and stir again; add 2 drops of (*d*) and stir the whole thoroughly. In presence of bismuth an orange-brown precipitate will be observed, whilst in absence of bismuth a yellow precipitate of lead iodide is formed. If the amount of reagent (*b*) is just insufficient to destroy all the nitrous acid formed a slow liberation of iodine discolours the drop, simulating the bismuth reaction; the two drops of (*d*) added completely destroy the liberated iodine, leaving the lead iodide of a bright yellow colour if bismuth is absent.

Tried on: Samples Nos. 8, 9, 14, 15, 16—Bismuth present; all gave positive results.
Nos. 2, 6, 10, 13, 18, 20, 22, 30, 31, 36—Bismuth absent and all results negative.

SUMMARY

Tests are described for the detection of alloying elements in lead-base alloys. The alloying elements so detected are tin, antimony, cadmium, silver, arsenic and bismuth.

With the alloys at our disposal the tests are specific and unambiguous. The amounts of tellurium present in commercial alloys are too low to permit a spot test.

Thanks are due to the Chief Scientist, Ministry of Supply, for permission to publish this paper.

APPENDIX

SPECIMENS OF ALLOYS USED IN THE TESTS. CONSTITUENTS PER CENT.

No.	Mark	Sn	Sb	Bi	Cd	As	Ag	Te	Remarks
1	EX.18	—	2·38	—	—	0·55	—	—	
2	EX.19	—	2·71	—	—	0·97	—	—	
3	EX.20	—	—	—	0·18	—	—	—	
4	EX.27	—	—	—	1·07	—	—	—	
5	EX.28	—	—	—	0·65	—	—	—	
6	EX.29	—	2·0	—	2·10	—	—	—	
7	EX.30	0·50	2·0	—	1·11	—	—	—	
8	EX.31	—	14·46	15·0	—	—	—	—	
9	EX.32	—	7·40	8·17	—	—	—	—	
10	EX.33	32·25	—	—	—	—	—	—	
11	EX.34	—	0·50	—	0·28	—	—	—	
12	EX.35	1·50	—	—	0·26	—	—	—	
13	EX.36	—	12·0	—	—	—	—	—	
14	EX.37	—	—	3·60	—	—	—	—	
15	EX.38	5·46	11·83	4·77	—	—	—	—	
16	EX.39	—	11·50	4·20	—	—	—	—	
17	EX.40	36·80	0·42	—	—	—	—	—	
18	EX.41	42·45	2·64	—	—	—	—	—	
19	EX.42	31·55	1·46	—	—	—	—	—	
20	EX.44	10	20	—	—	—	—	—	Nominal composition only
21	EX.45	5	10	—	—	—	—	—	
22	EX.57	—	—	—	—	—	2·48	—	
23	EX.58	0·95	—	—	—	—	1·36	—	
24	EX.59	4·90	—	—	—	—	1·36	—	
25	EX.60	20·05	9·43	—	—	—	0·53	—	
26	EX.61	24·50	1·52	—	—	—	0·52	—	
27	EX.62	29·22	—	—	—	—	0·57	—	
28	EX.63	31·93	1·77	—	—	—	0·98	—	
29	EX.64	36·16	1·86	—	—	—	1·11	—	
30	EX.65	43·67	—	—	—	—	1·39	—	
31	EX.68	—	—	—	—	—	—	0·068	0·007 per cent. Cu also present
32	EX.74	—	1·00	—	—	—	—	—	Nominal composition only
33	EX.75	—	0·50	—	—	—	—	—	
34	EX.76	—	0·25	—	—	—	—	—	
35	EX.77	25	—	—	—	—	—	—	
36	LEAD	—	—	—	—	—	—	—	

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Notes

THE TITRATION OF MINUTE AMOUNTS OF NICKEL*

AMENDMENT TO THE METHOD PUBLISHED IN "THE ANALYST," 1946, 71, 457

SINCE the publication of this method considerable trouble has arisen in the accurate titration of minute amounts (less than 0·0001 g.) of nickel, high results being obtained. This trouble has been traced to the extraordinary impurity of the amyl alcohol available. As amyl alcohol appears to be a very unreliable substance search was made for a substitute, and it appears that benzene is completely satisfactory.

The following figures were obtained by the same method but with 20 ml. of benzene instead of the original 20 ml. of amyl alcohol. The remaining procedure was the same, *i.e.*, use of

(*a*) 20 ml. of 20 per cent. ammonium nitrate solution.
(*b*) water up to 100 ml.
(*c*) 50 ml. of *N*/5 sodium carbonate (in view of the low amounts).
(*d*) 0·3 ml. of 1·5 per cent. diphenylcarbazone reagent.
(*e*) violent shaking for 15 seconds after each addition and occasional shaking of the blank.
(*f*) a stand of some minutes before the final matching.

Nickel taken mg.	Titration with 100-fold diluted KCN solution ml.	Nickel found mg.
0·0100	1·0	0·0105
0·0200	1·9	0·0200
0·0300	3·0	0·0315
0·0400	3·8	0·0399
0·0500	4·8	0·0504
0·0020	0·2	0·0021

The factor of the undiluted potassium cyanide solution was 1·050. The amount of nickel used in the final titration (only 2 μg.) was lower than anything I had attempted before.

Thanks are due to the Chief Scientist, Ministry of Supply, for permission to publish this note.

B. S. EVANS
January, 1947

A SIMPLE METHOD FOR ELECTRIC HEATING

HAVING had occasion to convert some incubators from gas to electric heating, I have found that ordinary mica-insulated electric-iron elements, which are cheap and easily obtainable, are very suitable for the purpose. The method used is to hold them firmly against the water-jacket by means of a piece of asbestos millboard in which slits are cut to take the electrodes. These are soldered to leads, another layer of millboard is placed over the leads, and the whole pressed in contact with the tank by tiles held in a wood or metal framework. I find that for an ordinary incubator or thermostat three, four or even five such units *in series* gives a convenient current (four in series passes about half an ampere). A series-parallel switch gives larger currents for starting-up, if necessary. Working thus with a small load, the life of the elements should be very long.

In these days of delay in getting things done through the usual channels this simple method may have a number of applications in laboratory heating problems.

H. S. HOWES
December, 1946

THE LABORATORY
4, QUEEN SQUARE, BRISTOL, 1

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* Communication from the Armament Research Department, formerly Research Department, Woolwich.

EVAPORATION FROM BEAKERS WITH RADIANT HEATER

THE application of the "Vitreosil" radiant heater to evaporation from open dishes is well known. Occasions arise, however, when it is convenient to complete an evaporation in a beaker, under conditions when heating from the top is necessary to avoid spitting. Under ordinary conditions this is very slow, but I have found that it is rapidly accomplished by placing the beaker or beakers in a metal vessel considerably larger than the beaker. Five squat-form 250-ml. beakers can be very conveniently heated simultaneously in this way by placing them under the heater in an aluminium saucepan 9 inches in diameter and 5 inches deep.

H. S. HOWES
THE LABORATORY
4, QUEEN SQUARE, BRISTOL, 1

December, 1946

Ministry of Food

STATUTORY RULES AND ORDERS

1947—No. 162. The Meat Products and Canned Meat (Control and Maximum Prices) Order, 1947. Dated January 30, 1947. Pp. 18. Price 5d.

This is a consolidating Order which substantially re-enacts the Meat Products, Canned Soup and Canned Meat (Control and Maximum Prices) Order, 1946; *S.R. & O., No.* 1355 *of* 1946; ANALYST, 1946, **71**, 382) *as amended* (*S.R. & O., No.* 1727 *and No.* 2046 *of* 1946; ANALYST, 1947, **72**, 64). *The principal changes are in maximum prices and charges. The restriction on labelling specified foods canned for sale to the Minister of Food has been removed.*

Meat paste and fish paste are not now listed in Schedule I, which is headed "Minimum Meat Content of Specified Foods (not Canned)"; but in Article 10 *the minimum meat content of meat paste is fixed at* 55 *per cent.* (50 *per cent. at discretion, in Court proceedings*) *and the minimum fish content of fish paste is fixed at* 70 *per cent.* (65 *per cent. at discretion, in Court proceedings*).

In accordance with S.R. & O., No. 2046 *of* 1946, *soups are not now included in the scope of the Order.*

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Seed Oils of some Sudan Caesalpinioideae. D. N. Grindley (*J. Soc. Chem. Ind.*, 1946, **65**, 118–119)—Fatty acids of high molecular weight (20 to 24 carbon atoms) are characteristic of Caesalpinioideae, as of the other Leguminosae sub-families. Constants were determined for the following plants: (i) *Erythrophleon guineense*, a well known poison tree containing as main active principle the alkaloid erythrophleine, $C_{28}H_{43}O_7N$, (ii) *Parkinsonia aculeata*, (iii) *Cassia fistula*, of the British Pharmacopoeia, (iv) *Tamarindus indica*, of the British Pharmacopoeia, and (v) *Cassia arereh*, a small tree of the Fung area. Results obtained are: Saponification values, (i) 190·4, (ii) 190·6, (iii) 184·9, (iv) 181,9, and (v) 192·3; unsaponifiable matter, (i) 2·5, (ii) 6·25, (iii) 5·4, (iv) 3·8, and (v) 2·7 per cent.; n_D^{40}, (i) 1·4661, (ii) 1·4719, (iii) 1·4668, (iv) 1·4667, and (v) 1·4672; iodine values, (i) 98·1, (ii) 114,0, (iii) 94·5, (iv) 94·5, and (v) 93·8; thiocyanogen values, (i) 63·1, (ii) 69·1, (iii) 63·2, (iv) 61·3, and (v) 61·5; m.p. of fatty acids (slip point), (i) 32·5, (ii) 33, (iii) 37, (iv) 45·5 (titre 43°) and (v) 33·5° C. Insoluble fatty acids plus unsaponifiable matter (Hehner value) are (i) 96·1, (ii) 96·3, (iii) 96·05, (iv) 95·6, and (v) 95·3 per cent. The approximate percentage compositions of the mixed fatty acids are: Oleic, (i) 26·8, (ii) 20·6, (iii) 31·9, (iv) 27·3, and (v) 28·7; linoleic, (i) 43·6, (ii) 56·0, (iii) 39·0, (iv) 41·9, and (v) 40·2; saturated acids, (i) 29·6, (ii) 23·4, (iii) 29·1, (iv) 30·8, and (v) 31·1; higher saturated acids, (C_{20} to C_{24}), (i) 1·6, (ii) 1·0, (iii) 4·8, (iv) 11·8, and (v) 4·4. The oil contents of the seeds are (i) 5·60, (ii) 1·65, (iii) 2·04, (iv) 4·33, and (v) 5·54 per cent. All the oils are highly coloured. The higher acids of (iv) had mean molecular weight of 374 and m.p. 78·5° C., and are thought to be mainly lignoceric acid. E. B. D.

Polarographic Determination of Tin in Foods and Biological Materials. E. M. Godar and O. R. Alexander (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 681–684)—The method is accurate to about ±5 per cent. for concentrations of tin greater than 10 p.p.m., but is applicable to concentrations as low as 0·5 p.p.m. Much smaller samples than in the iodimetric method (A.O.A.C., "*Official and Tentative Methods of Analysis,*" 5th Ed., 1940, p. 413) are used. Since a mercury pool anode causes reduction of stannic tin, an external saturated calomel reference electrode is used. The design of the polarographic cell permits nitrogen to be passed either through or over the surface of the solution.

Method—Take 5 to 10 g. of sample, add 10 to 15 ml. of nitric acid and 2 to 3 ml. of sulphuric acid. Heat slowly, adding small amounts of nitric acid as charring occurs. When fumes of sulphur trioxide are evolved with no further darkening or charring, add 1 ml. of perchloric acid and continue heating until the latter has volatilised. Allow to cool, add 10 to 15 ml. of water, and transfer to a 50-ml. conical centrifuge tube, washing in with a little water and finally diluting to approximately 20 ml. Add 1 ml. of aluminium chloride solution (4·65 g. of the nonahydrate [? hexahydrate.—Ed.] in 250 ml. of water; 1 ml. ≡ 2 mg. of Al), and 1 drop of methyl red. Make alkaline with concentrated aqueous ammonia, adding 0·1 to 0·2 ml. in excess, but avoid a large excess. Centrifuge at high speed and decant the clear liquid as completely as possible. Add to the residue 2·5 ml. of hydrochloric acid (1+1) and shake to dissolve. Dilute to 10 ml. with saturated ammonium chloride solution. Transfer a 4- to 5-ml. portion to the cell and add 1 drop of saturated cresol red solution. Bubble nitrogen through the solution for 10 min., then divert the gas to blanket the surface. Record the polarogram between 0 and 0·8 v. at the highest sensitivity permitting the recording of the complete curve. Measure the height of the second tin wave and calculate the tin content of the sample from a calibration curve prepared as follows. Transfer aliquots of standard tin solution (dissolve 0·5 g. of tin in 250 ml. of hydrochloric acid and dilute to 500 ml.; dilute 25 ml. to 250 ml. with hydrochloric acid (1+1): 1 ml. = 0·1 mg. of Sn) to centrifuge tubes, dilute, and precipitate as described above. Record the polarograms, measure the wave heights, and plot the latter against the concentrations of tin. Run additional standards at intervals.

Iron, copper, bismuth, cadmium, mercury, antimony, nickel, cobalt, and zinc in equal or slightly greater concentrations do not interfere, but lead is precipitated with tin, and is reduced at a voltage too near to that of the second tin wave to be resolved. The interference is not serious in the analysis of foods, in which the lead concentration is relatively minor. When significant amounts of lead are suspected, proceed as follows. Record the polarogram as usual, and measure the combined wave height due to tin and lead. To a 5-ml. aliquot add 1 ml. of concentrated aqueous ammonia and 0·5 ml. of ammonium citrate solution (50 per cent. w/v). Record the polarogram and measure the height of the lead wave (tin is completely suppressed). Prepare tin-free solutions containing known amounts of lead, and precipitate in the presence of aluminium as above. Dissolve and take a polarogram in the supporting electrolyte of hydrochloric acid and ammonium chloride. Repeat after addition of ammoniacal citrate. Plot the lead wave heights in acid medium against those in alkaline medium. Use the curve to convert the height of the lead wave given by the sample in alkaline medium to the equivalent height in acid medium. Deduct this computed wave height from the combined wave height of tin and lead to obtain that due to tin.

J. T. S.

Biochemical

Simplified Method for the Determination of Sodium [in Biological Fluids]. J. T. Bradbury (*J. Lab. Clin. Med.*, 1946, **31**, 1257–1261)—In an investigation of the ability of various diuretics to cause excretion of sodium, it became necessary to develop a method for the rapid determination of that metal in urine. The uranyl zinc acetate procedure of Barber and Kolthoff (*J. Amer. Chem. Soc.*, 1928, **50**, 1625; 1929, **51**, 3233) as modified by Butler and Tuthill (*J. Biol. Chem.*, 1931, **93**, 171) and by Salit (*Ibid.*, 1932, **96**, 659) has been simplified by measuring the colour of the supernatant fluid after the precipitation of the triple acetate, the reduction in colour being found to be proportional to the amount of sodium present originally.

Procedure—Transfer 1 ml. of clarified urine, deproteinised serum, or other sodium-containing fluid, which might be expected to contain between 1 mg. and 10 mg. per ml. of the metal, to a 15-ml. centrifuge tube, add exactly 4 ml. of freshly filtered uranyl zinc acetate reagent and 2 ml. of 95 per cent. alcohol, and mix throughly. The reagent should be prepared according to the method of Weinbach (*Ibid.*, 1935, **110**, 95), or by dissolving 77 g. of uranyl acetate in a mixture of 800 ml. of water and 14 ml. of glacial acetic acid, adding 281 g. of zinc acetate in small quantities, then 7 ml. of glacial acetic acid together with sufficient water to produce nearly 1 litre and, finally, after leaving overnight, diluting to exactly 1 litre. If urine is being examined, a control solution should be prepared as above except that 4 ml. of water are substituted for the uranyl zinc acetate reagent. Centrifuge the mixture, decant the clear supernatant fluid into the absorption cell of a photo-electric colorimeter, and determine the intensity of the colour, using a blue filter with a Fisher electrophotometer; 3-ml. micro-cells are specified and a blue filter No. 425 is employed. Subtract the photometer reading of the blank from that of the test, and correlate the value found with the quantity of sodium by reference to a graph constructed from data obtained by submitting standard solutions of sodium chloride to the above procedure.

The method has been checked by comparison with results obtained gravimetrically; satisfactory agreement was obtained. The urine should be prepared for the test by allowing ammonia to bubble through the sample until it is strongly alkaline. It should be set aside in a refrigerator for several hours, and then filtered. Albumen may be removed, if present, by adding mercuric chloride or trichloroacetic acid. Blood or serum may be deproteinised by mixing with an equal volume of 20 per cent. trichloroacetic acid, stirring vigorously, and removing the precipitate by centrifuging. J. A.

Estimation of Cobalt in Biological Materials with Nitrosocresol. G. H. Ellis and J. F. Thompson (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 254–257)—*o*-Nitrosophenol forms coloured complex compounds with many metals, but only those of ferric iron, palladium, and cobaltous cobalt are soluble in light petroleum. With most plant and animal tissues only the interference due to iron must be eliminated in order to give a colorimetric cobalt determination. Where the cobalt content is very small relative to the iron, interference is not completely avoided by use of a citrate buffer, but separation can be achieved by using sodium diethyldithiocarbamate or, more generally, dithizone. *o*-Nitrosocresol is preferable in that it is more easily prepared than *o*-nitrosophenol and produces a more intense colour with cobalt. The method described is suitable for use with 1 to 10 g. of dry animal or plant tissue containing 0·02 to 25 µg. of cobalt.

REAGENTS—(1) *Stock cupric o-nitrosocresol solution*—Dissolve 6 g. of hydroxylamine hydrochloride and 15 g. of cupric chloride in 900 ml. of water, and add 5 ml. of *m*-cresol. Stir in 15 ml. of 30 per cent. hydrogen peroxide. After 2 hr., add 25 ml. of concentrated hydrochloric acid and extract the reagent with successive portions of light petroleum. Wash the extract with water and 50- to 100-ml. portions of 1 per cent. aqueous cupric acetate until the upper layer is colourless. The deep-red, aqueous solution of the cupric complex is then stored in a refrigerator; it is stable for months.

(2) *Sodium borate buffer*—Dissolve 20 g. of boric acid in 1 litre of water and add 22·8 ml. of 1·0 *N* sodium hydroxide to give *p*H 7·7 to 7·8.

(3) *Sodium nitrosocresol solution*—Add 10 ml. of concentrated hydrochloric acid to 75 ml. of the stock copper-complex solution and shake with 300 to 500 ml. of light petroleum. Wash this phase with two 100-ml. portions of 0·01 *N* hydrochloric acid and two 100-ml. portions of re-distilled water. Add 25 ml. of a borate buffer made by diluting 1 litre of the borate solution, prepared as above, with 20 ml. of *N* sodium hydroxide solution, and shake. Remove the buffer solution and repeat this treatment until the light petroleum layer is almost colourless; stored in a refrigerator the aqueous reagent is stable for about a month.

(4) *Carbamate solution*—Shake a 0·1 per cent. aqueous solution of sodium diethyldithiocarbamate with carbon tetrachloride to remove copper and cobalt.

(5) *Dithizone solution*—Dissolve 0·5 g. of dithizone in 600 to 700 ml. of carbon tetrachloride and filter into 2·5 to 3 litres of 0·02 *N* aqueous ammonia and shake; discard the non-aqueous layer. Shake then with 50-ml. portions of carbon tetrachloride until a pure green layer settles after standing. Add 1 litre of the tetrachloride and acidify slightly with hydrochloric acid; shake well, separate, and store the solution in a cool, dark place.

(6) *Hydroxylamine-acetate buffer*—Dissolve 10 g. of hydroxylamine hydrochloride and 9·5 g. of sodium acetate (anhydrous) in 500 ml. of re-distilled water; the *p*H of the solution should be 5·0 to 5·2.

(7) *Potassium nitrate solution*—Shake a 5 per cent. solution of potassium nitrate with 0·1 per cent. aqueous carbamate solution and carbon tetrachloride.

(8) *40 per cent. ammonium citrate solution*—Dissolve 800 g. of citric acid in 600 ml. of water and add slowly 900 ml. of concentrated aqueous ammonia. Adjust to *p*H 8·5, if necessary. Dilute to 2 litres and extract with 10-ml. portions of dithizone in carbon tetrachloride until the aqueous phase remains orange-coloured. Then extract with tetrachloride until the orange colour is removed.

(9) *Ligroin [light petroleum], b.p.* 70 *to* 90° C.—Distil over dilute alkaline permanganate and wash by vigorous shaking with water.

(10) *Standard cobalt solution*—Dry cobalt sulphate heptahydrate at 250 to 300° C. for 6 to 8 hr., and dissolve 0·263 g. in 50 ml. of re-distilled water, and 1 ml. of sulphuric acid. Dilute to 1 litre, and dilute further as required.

(11) *Ammonia solution*—Distil concentrated aqueous ammonia into an equal volume of re-distilled water.

(12) *Phenolphthalein*—A 1 per cent. solution in 95 per cent. ethanol.

(13) *Bromothymol blue*—A 0·04 per cent. aqueous solution of the sodium salt.

Reagent grade perchloric acid (60 or 72 per cent.), redistilled hydrochloric acid (1+1), and nitric acid (1+1) are also required. Also distil carbon tetrachloride over lime and filter through acid-washed paper. All distillations must be done in Pyrex apparatus.

METHOD. *Preparation of sample*—Tissues may be ashed by the usual methods, nitric acid and potassium nitrate being used, if necessary. High silica contents should be removed with hydrofluoric acid and perchloric acid; otherwise adsorption is reduced by heating the ash solution for several hours before neutralising.

Separation of cobalt from iron. Dithizone extraction—Add 1 ml. of ammonium citrate for each gram of dry tissue used and adjust the *p*H to 8·5 by means of aqueous ammonia (phenolphthalein). Shake for 30 sec. with 10 ml. of dithizone solution, repeating the extraction if necessary, so that the green coloration is unchanged. Evaporate the extracts to dryness. *Carbamate extraction*—Proceed as above, but adjust the *p*H to 6·5 (bromothymol blue). Add dropwise with shaking 5 ml. of the 0·1 per cent. solution of the reagent and 10 to 20 ml. of tetrachloride, and shake vigorously for 10 min. Evaporate this extract, which also contains any copper present, to dryness.

Determination of cobalt—Oxidise the residue obtained from either extraction with 2 ml. of perchloric acid in a covered beaker. When the solution is colourless, remove the perchloric acid, and add 5 ml. of 0·01 *N* hydrochloric acid and 5 ml. of the borate buffer solution. Transfer to a 60-ml. separating funnel and add sodium nitrosocresol solution dropwise until reaction with the copper is complete, as shown by the appearance of an orange colour. Add 1 ml. of the reagent in excess, then 4 to 5 ml. of ligroin and shake for 5 min. Remove the aqueous phase, and to the ligroin add 5 ml. of 1 per cent. aqueous cupric acetate solution. Shake for 1 min. and again separate; wash the ligroin layer with water and finally with 5 ml. of the hydroxylamine-acetate buffer. Unless much cobalt is present the solution is colourless, absorption occurring in the near ultra-violet. Transfer to a 5- or 10-cm. absorption tube and read in a photo-electric colorimeter using a light band near 360 mμ, where maximum absorption occurs.

Blank determinations should be carried out, especially where conditions require a large deviation from the usual procedure, using either the blank, or pure ligroin as the reference liquid. Since Beer's law holds over a wide concentration range, calibration is relatively simple. In a 10-cm. tube, 0·01 μg. of cobalt per ml. transmits about 90 per cent. of incident light in the recommended wave-band.

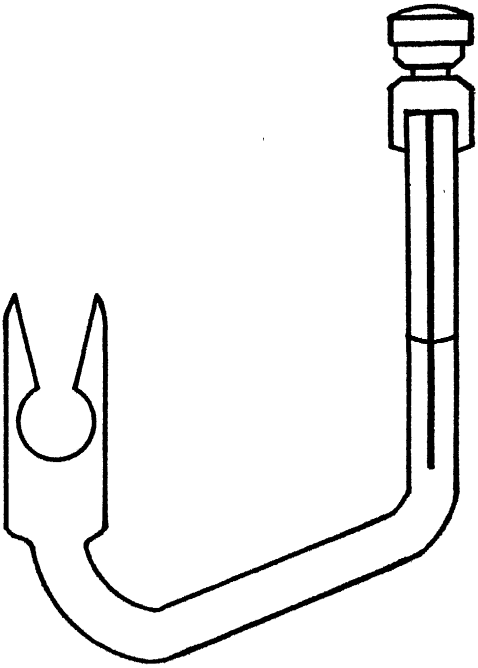
Results—With cobalt contents of about 0·03 μg. the range of recovery is from 96 to 107 per cent., and with contents of about 0·2 μg., from 89·0 to 104·5 per cent. An average on 20 determinations is 97·4 per cent. with determinations in both ranges. The values agree well with those obtained by the nitroso-R-salt method.

Discussion—Cobalt nitrosocresol is determined in a smaller volume of solution and the test is therefore more sensitive than that with the nitroso-R-salt. Ashing at 650° C. prevents adsorption of copper and iron on the silica vessel and the results for cobalt are not affected unless the vessel is badly etched. The use of the hydroxylamine-acetate buffer causes the ferric complex to be reduced to a form soluble in the aqueous phase, but is not effective in presence of more than a ten-fold excess of iron. Dithizone removes all ferrous iron and is widely applicable, whilst carbamate is useful in that it reacts at a lower *p*H and thus is less likely to precipitate any salts present; also, a single extraction is often sufficient. Copper can be determined in the same extract since the cobalt to copper ratio is usually small, and the copper coloration is not altered. Copper may also be determined after removal of the aqueous phase from the ligroin solution of the cobalt-complex, either directly or after first evaporating the solution to dryness with 1 ml. of perchloric acid. M. E. D.

Organic

System of Characterisation of Pure Hydrocarbons. Refractometric Analysis as a Key to the Evaluation of Structure. R. T. Wendland (*J. Chem. Educ.*, 1946, **23**, 3–15)—A system for the classification of hydrocarbons according to several of their physical properties, and according to their response to a few simple chemical tests, is the basis of a method for determining the nature of these compounds. The properties required are boiling point, density, refractive index, specific dispersion, and specific refraction. By comparison of the properties of an unknown hydrocarbon with the chemical properties and the limiting values of the physical properties recorded in the various classes, divisions, and groups of the table given in the paper, the compound can be shown to be a member of one particular sub-group. To identify the compound, it is then necessary to distinguish it from a small number of closely related compounds. A further comparison of properties and, when possible, the preparation of derivatives, will usually provide strong evidence in favour of one of the possible structures. In order to exemplify the working of the new scheme of classification, various hydrocarbons chosen at random from the literature were correctly placed in the sub-groups having properties corresponding to their properties, the structure of each hydrocarbon chosen being included in the structures listed in the sub-group of the classification. B. A.

Micro-titration of Organic Acids. W. Ingold (*Helv. Chim. Acta*, 1946, **29**, 1929–1936)—A potentiometric method is used. The re-entrant glass electrode shown also acts as titration vessel; the bulb has a capacity of about 1·5 ml. A suction pipette, the tip of which is plugged with cotton wool, enables the vessel to be emptied without danger of puncturing the glass membrane. The calomel reference electrode has a fine, hooked outlet which may be flushed out with potassium chloride solution by the operation of a tap. The micro-burette is of horizontal, tapless form (*cf.* Hybbinette and Benedetti-Pichler, *Mikrochem.*, 1942, **30**, 15) with a paraffined jet, has a capacity of about 0·15 ml., and can be read to 0·0001 ml. It is filled by suction and has a soda-asbestos guard tube to prevent ingress of carbon dioxide. A lever mechanism enables the burette tip to be immersed in the test solution for delivering. Potassium hydroxide and tetramethylammonium hydroxide solutions (not less than 0·1 *N* and free from carbonate) are preferred as titrants, since the alkali error of the glass electrode is less than with sodium or lithium hydroxides. The test solution is stirred by a stream of carbon-dioxide-free nitrogen.



Before introducing the sample, rinse the titration vessel and dry the conical neck, first with a cotton plug and then with chamois leather. Add the sample (300 to 900 μg.); boiled-out distilled water or alcohol-water mixtures are the usual solvents. The bulb of the vessel should be not more than two-thirds full. Arrange the tips of the reference electrode and gas inlet tube to dip into the solution, and stir by passing 2 to 3 bubbles per sec. Clamp the micro-burette so that operation of the lever immerses the burette tip 1 to 2 mm. in the solution, connect to a suitable *p*H meter, and titrate. The error is ± 5 per cent. with organic acids of molecular weight 100 to 500. J. T. S.

Conversion of Organic Sulphur to Hydrogen Sulphide for Analysis. E. Field and C. S. Oldach (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 668–669)—Since many catalytic processes are affected by traces of hydrogen sulphide or organic sulphur, sensitive analytical methods are necessary to determine and eliminate them. Satisfactory methods of determining hydrogen sulphide are available, *e.g.*, the Field and Oldach colorimetric method (*Ibid.*, 1946, **18**, 665) also the Moses and Jilk lead-acetate-impregnated tape method (U.S. Patent 2,232,622, Feb. 18th, 1941). The following technique involves the conversion of volatile organic sulphur compounds to hydrogen sulphide, which may then be determined by any suitably accurate method. Jilk's procedure (*Proc. Amer. Soc. Testing Materials, Preprint* 89, 1939) has been modified by raising the reaction temperature to 900° C., thus rendering moistening of the gases unnecessary, and also allowing an oxygen content of up to 1 per cent. without affecting the results.

Procedure for gases—Pass the gas with an excess of hydrogen over 6 ml. of 14- to 20-mesh alumina in a 10 mm. I.D. quartz tube heated to 900° C. All connecting lines must be of glass and, where they are unavoidable, rubber joints must first be boiled with sodium hydroxide to remove sulphur. Maintain a gas rate not exceeding 0·6 litre per min. Taking precautions to reduce errors due to adsorption, absorb the emerging gases in 6 per cent. sodium hydroxide solution in a Milligan bubbler.

Procedure for liquids—Pass sulphur-free hydrogen at the rate of 0·6 litre per min. through the liquid sample contained in a warmed bubbler, adjusting the volume and temperature of the sample to give a convenient sulphur content and rate of evaporation. Pass the gases then over heated alumina as before, absorbing the emergent gases in sodium hydroxide, until the weighed sample is completely volatilised. Take the same precautions against adsorption as with gaseous samples. With high-boiling liquids, it is necessary only to strip off the low-boiling sulphur constituents. Deposition of carbon on the catalyst lowers its activity and increases adsorption; hence the catalyst must be regenerated by passing air over it at 900° C. for 5 min., and purging with hydrogen before each regeneration. If this is effected during an analysis, the air must be by-passed round the absorbing solution to prevent oxidation of the sulphide.

Demonstration of conversion to hydrogen sulphide—Using 14- to 20-mesh alumina at 900° C., and a carrier gas of pure hydrogen or water gas and introducing a measured volume of vapour into the stream, hydrogenation was demonstrated for carbonyl sulphide, carbon disulphide, methyl mercaptan, methyl isocyanate, thiophen, and sulphur dioxide. Errors of up to ±4 per cent. on pure compounds were attributed to experimental inaccuracies.

Adsorption of sulphur on catalyst—With a fairly uniform gas sample, adsorption errors are reduced by allowing the catalyst to come to equilibrium with the gas before starting an analysis. With batch samples and liquids, the catalyst must be purged with pure hydrogen before and after treating the sample, 28 litres being necessary to remove all the sulphur from 6 ml. of 8- to 14-mesh alumina at 900° C.

The method is widely applicable, and traces of sulphur have been determined in hydrogen, carbon monoxide, methane, ethylene, nitrogen, and coke-oven gas; methanol, benzene, *cyclo*hexane, tetralin, and white oil. The presence of water-vapour is not essential, but it often reduces carbon deposition.

Carbon dioxide does not interfere unless in sufficient quantity to deteriorate the absorbing solution Unsaturated compounds are not deleterious. Oils and tars forming in the scrubber may affect results, but any absorbed hydrogen sulphide can then be liberated by acidification and again absorbed in alkali. M. E. D.

Inorganic

Method of Semi-Quantitative Inorganic Analysis. P. E. Wenger, D. Monnier, and A. Piguet (*Helv. Chim. Acta*, 1946, 29, 1698–1701)—On the basis of the tests recommended in the second Report of the International Commission (of the Union Internationale de Chimie) on new analytical reagents and reactions, it should be possible to establish a series of rapid semi-quantitative tests for each element. It is intended to examine this possibility element by element. This is a preliminary report, in which details are given of an estimation of copper based on the limit of perceptibility of the test given by copper with several reagents. Reagents chosen for this purpose must be sufficiently selective, and have as great a difference of sensitivity as possible. Tables are prepared that give directly, from the limit of perceptibility, the percentage of the element in the sample being examined.

Reagents—A 1 per cent. solution of rubeanic acid in 96 per cent. alcohol, a 0·1 per cent. solution of sodium diethyldithiocarbamate in water, a 1 per cent. solution of potassium ethylxanthate in water, and a 1 per cent. solution of benzoinoxime in alcohol. Carry out the tests on filter papers impregnated with the reagent and dried.

Method—Prepare solutions containing, A, 0·1 g.; B, 0·004 g.; and C, 0·002 g. of copper per litre. On a spot plate place 1 drop of A and 9 drops of water to give A_1, 2 drops of A and 8 drops of water to give A_2, and so on to . . . . 10 drops of A to give A_{10}. Similarly, prepare B_1 to B_{10} and C_1 to C_{10}. Determine which are the most dilute solutions that give perceptible tests with the test papers of the four reagents. To estimate the copper in a substance, take exactly 0·1 g. of sample, dissolve it in the minimum quantity of a suitable solvent, and dilute to 1 litre to obtain solution A^e. Prepare from this solution A^e_1 to A^e_{10}, B^e_1 to B^e_{10}, C^e_1 to C^e_{10}. Find the limits of perceptibility as before. Prepare a table showing the percentage of copper corresponding to the possible positions of the limit for each reagent. For rubeanic acid, the limit found by using the standard solutions is C_3, and if the limit in the estimation is C^e_3 the sample is 100 per cent. copper; if it is C^e_4, 75 per cent. copper; and if A^e_1, 6 per cent. of copper. From the table and the limit found, deduce the amount of copper in the sample.

For a copper content of 2 to 10 per cent. the result is accurate to ± 1 per cent., and for higher contents to ± 5 per cent. Two examples are given of the application of the method to the estimation of copper in alloys. If the sample dissolves without great difficulty, the estimation can be completed in 30 min. B. A.

Sensitivity of Precipitation Reactions in Dilute Electrolytes. R. K. McAlpine (*J. Chem. Educ.*, 1946, 23, 28–34)—Factors considered in this discussion of the sensitivity of the lead chloride, lead iodide, and lead chromate precipitation tests for lead are solubility, the common ion effect, supersaturation, and visibility of the precipitate.

When the sensitivities of the tests for lead, in which lead chloride or lead iodide is precipitated from aqueous solutions, are examined, it is found that both tests are less sensitive than is indicated by calculations based on the solubility product principle. The discrepancies are not due to the formation of supersaturated solutions, nor to inability to observe the precipitate, but are caused partly by inter-ionic attraction and partly by the formation of ions such as $PbCl^{\cdot}$ and $PbI^{\cdot}$. It is probable that the reduced sensitivity of the lead iodide test in the presence of perchloric acid is the result of inter-ionic attraction, whilst the reduced sensitivity of the same test in the presence of chloride ions is due to the formation of $PbCl^{\cdot}$. From measurements now made of the sensitivity of the lead chloride and lead iodide tests in the presence of an excess of potassium chloride and potassium iodide, respectively, and known data on the solubilities of the lead salts in water, assuming that lead chloride and lead iodide are completely ionised in aqueous solution, the apparent values of the dissociation constants of the ions $PbCl^{\cdot}$ and $PbI^{\cdot}$ can be calculated. The value for the constant for $PbCl^{\cdot}$ appears to lie between 0·05 and 0·01, and that for $PbI^{\cdot}$ between 0·005 and 0·001. However, observations made on the precipitation of lead iodide by an excess of lead perchlorate indicate a value between 0·05 and 0·01 for the dissociation constant of $PbI^{\cdot}$. When an excess of potassium iodide is used for the precipitation of lead iodide, the formation of complex ions such as PbI_4'' may have a significant effect on the solubility of the lead iodide. If the reagent in excess is lead perchlorate, the possibility of the formation of ions other than those considered in the calculations is less, and therefore the second value for the dissociation constant of $PbI^{\cdot}$ is probably the more correct. Because of small effects due to supersaturation and inter-ionic attraction, this value will be slightly low. The marked reduction in the sensitivity of the lead iodide precipitation test that occurs when nitrate ion is added, can be explained by the existence of the ion $PbNO_3$, with a dissociation constant greater than that of $PbCl^{\cdot}$.

The solubility of lead chromate, in the presence of excess chromate, is negligible compared with the amount of precipitate, 0·01 to 0·02 mg. of lead in 50 ml. of solution, required to produce a recognisable opalescence. Calculations based on solubility product give the correct order of the sensitivity of the test when it is carried out in the presence of perchloric acid. B. A.

Colorimetric Determination of Fluoride. D. Monnier, Y. Rusconi, and P. Wenger (*Helv. Chim. Acta*, 1946, **29**, 521–525)—The soluble, violet-coloured sulpho-5-salicylic acid ferric complex is decolorised by fluorides because of the formation of the more stable FeF_6''' ion. As Beer's Law is not obeyed, calibration curves must be used, and they are prepared by adding known amounts of fluoride to a solution of the complex, and determining the extinction, E, in a Pulfrich photometer using a number 4 filter, and plotting K (E/length of tube in centimeters) against fluoride concentration. The ferric sulphosalicylate complex is stable in an excess of the acid in the absence of fluoride. The fading is not solely dependent on the fluoride concentration, although this is the chief factor, and the colour tends to return. Reproducible results are obtained if sufficient sulphosalicylic acid is present to give the maximum intensity of coloration of the complex. This value was determined by investigating the composition of the complex, which was found to contain one molecule of the acid to one ferric ion as compared with the ferric salicylate complex which contains two molecules of salicylic acid to each ferric ion.

Calibration solutions—(A) 2 ml. of 0·11 per cent. ferric chloride solution, 0·8 ml. of *N* ammonium chloride, 2 *N* hydrochloric acid to *p*H 3, and 0·75 ml. of 0·5 per cent. sulphosalicylic acid solution, the mixture being diluted to 20 ml.

(B) 5 ml. of 0·11 per cent. ferric chloride solution, 2 ml. of *N* ammonium chloride, 2 *N* hydrochloric acid to *p*H 3, 1·9 ml. of 0·5 per cent. sulphosalicylic acid solution, diluted to 25 ml.

(C) 10 ml. of 0·11 per cent. ferric chloride solution, 2 ml. of *N* ammonium chloride, 2 *N* hydrochloric acid to *p*H 3, and 4 ml. of 0·5 per cent. sulphosalicylic acid solution, diluted to 25 ml.

The calibration curves are prepared by adding known quantities of 0·2 per cent. sodium fluoride solution to these solutions and determining K, the sodium fluoride solution having been previously adjusted to *p*H 3 with a few drops of hydrochloric acid. As the fading action tends to decrease on keeping the solutions, the determinations should be made within 3 to 4 hr. The solutions are useful within the ranges; A—0·10 to 1·0 ml. of 0·2 per cent. sodium fluoride solution, B—0·25 to 3 ml. of the fluoride solution, and C—1·0 to 6·0 ml. of the fluoride solution.

Analyses show that the method is applicable to low fluoride contents; it gives a maximum error of 5 per cent.

Procedure—Take 5 ml. of 0·11 per cent. ferric chloride solution, 2 ml. of *N* ammonium chloride, and add hydrochloric acid to adjust the *p*H to 3. Add 1·9 ml. of 0·5 per cent. sulphosalicylic acid solution and a small volume of the fluoride solution to give a convenient fading. Dilute to 25 ml. Measure E, divide by the length of the tube in centimeters to obtain K, and refer to the abscissa of the appropriate calibration curve. The ordinate gives the fluoride content.

The effects of the presence of other ions, concentration changes, temperature, and dilution on the method are being investigated. M. E. D.

Constitution of Molybdenum Blue. W. D. Treadwell and Y. Schaeppi (*Helv. Chim. Acta*, 1946, **29**, 771–783)—Molybdenum blue, produced in the reduction of acid solutions of molybdates, is a combination of quinque- and sexavalent molybdenum oxides. The molecular proportions of these oxides vary with the method of preparation. As a large change in this ratio causes only a slight difference in percentage composition, composition has been determined oxidimetrically and conductometrically. *Oxidimetric method*—Electrically produced molybdenum blue hydrated oxide, precipitated in dilute sulphuric acid, was titrated in sulphuric acid solution by potassium permanganate to determine the quinquevalent molybdenum. The molybdenum of the titrated solution was reduced to the tervalent stage in a cadmium reductor, and the titration with permanganate repeated to determine the total molybdenum. The ratio of the two amounts of permanganate used clearly indicated the proportions of quinque- and sexavalent oxides, which were found to be $Mo_2O_5.4MoO_3$. *Conductometric method*—A solution of 500 mg. per litre of the electrolytic molybdenum blue was titrated conductometrically with 0·1 *N* sodium hydroxide, 100 ml. of the solution being used. For comparison, 100 ml. each of saturated molybdenum solution and 0·001 *N* sulphuric acid were similarly titrated. The acidity of the molybdenum blue was approximately that of molybdic acid. No appreciable amount of sulphuric acid was detected in the precipitate, and the acidity was attributed not to adhering sulphuric acid, but to acid hydroxyl groups. The results of these titrations, together with the molecular weight of 436 determined by the freezing point method, indicated that the aqueous solution of molybdenum blue hydrated oxide contained 1 hydrogen ion per $\frac{1}{2}$ $Mo_2O_5.2MoO_3$. A structural formula that explains the deep colour and the tendency to form negative sols, is proposed.

In sulphuric acid solution, molybdenum blue forms complex compounds with phosphoric and arsenic acids, as well as with sulphuric acid. Solutions of molybdenum trioxide in sulphuric acid and the same solution of molybdenum reduced to the tervalent state were mixed to give solutions containing molybdenum of mean valency 5, 5·1, 5·2 . . 6, the total concentration of molybdenum remaining constant. Colorimetric determinations showed the colour to be strongest if the mean molybdenum valency was 5·67 (curves are given). Phosphates were prepared from the above sulphates as follows: a series of the solutions, containing the same total amount of molybdenum, were placed in 50-ml. flasks, diluted with a known amount of water until decolorised, the same amounts of phosphate solution being added to all, and the mixtures were made up to the mark. They were then kept for 1 hr. in boiling water, and the blue colour was determined colorimetrically after (*a*) 24 hr., or (*b*) 50 hr. In the first tests, the molybdenum blue was in large excess; in others, the amount of phosphate was increased. Molybdenum blue arsenates were similarly prepared. The 24-hr. curves showed that the reduction proceeded by stages; the 50-hr. curves were smooth. The first tests on sulphate, phosphate, and arsenate all showed maximum colour strength when the mean molybdenum valency is 5·67, but with increasing amounts of phosphoric or arsenic acid the valency for maximum colour decreased; this was due to the formation of Mo^V phosphate or arsenate in a side reaction. For a given amount of molybdenum sulphate of optimum valency, increasing amounts of phosphoric acid increased the blue colour until the ratio $1PO_4:12Mo$ was reached. Pyrophosphate is similar to phosphate in the development of molybdenum blue, giving the same result. Sodium hypophosphate, $Na_2H_2P_2O_6$, in sulphuric acid solution gave a violet-blue colour very quickly in the cold; under the same conditions, the hypophosphate colour was much more intense than that of the phosphate. A molybdenum blue perchlorate could not be prepared. E. B. D.

Separation of Calcium from Magnesium by the Oxalate Method in Samples of High Magnesium - Calcium Ratio. E. R. Wright and R. H. Delaune (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 426–429)—The procedure in which a large amount of oxalate is employed to prevent the precipitation of magnesium oxalate and to ensure maximum precipitation of calcium has been investigated. Solubility data are given for calcium oxalate and magnesium oxalate in hot ammonium oxalate solutions of a range of concentrations. As the oxalate concentration is increased, the solubility of magnesium oxalate increases considerably, presumably owing to the formation of a complex ion. Three modes of precipitation have been examined. (A) Add 6 *N* aqueous ammonia dropwise to an acid solution at 70° to 80° C. containing 1·5 g. or more of ammonium oxalate monohydrate per 100 ml. until the liquid is alkaline to methyl red. Set aside for 1 hour without heating, and filter cold. When more than 5 g. of ammonium oxalate monohydrate are used, maintain the solution at 70° C. to prevent crystallisation of the reagent on cooling. (B) Add the sample solution dropwise to a hot ammonium oxalate solution of such a dilution that the final volume is 100 ml. Keep at 70° C. for 0·5 hr., and filter hot. (C) Heat 100 ml. of neutral sample solution containing approximately 1 g. of ammonium chloride to 80° C., add the amount of solid ammonium oxalate indicated in the table, stir until solution is complete, heat for 0·5 hr. at 80° C., and filter hot through a tared, fine porosity, glass filter crucible. Wash the precipitate with cold, 0·2 per cent. ammonium oxalate solution.

Method (A) gives more co-precipitation of magnesium than (B) or (C), but less precipitation of magnesium oxalate when insufficient ammonium oxalate is used. Method (B) is less convenient than (C), which gives almost equally good results. Methods (B) and (C) both give negligible co-precipitation of magnesium, but the result must be corrected for the solubility of calcium oxalate in the hot medium (see Table). Results of acceptable accuracy are obtained with magnesium - calcium ratios by weight up to 20:1 either by a single precipitation with method (C), using the correction factor; or by method (A) with not more than 5 g. of ammonium oxalate monohydrate and 0·2 g. of magnesium ion per 100 ml., a second precipitation of the calcium, cold filtration, and consequently no solubility correction.

RECOMMENDED QUANTITY OF AMMONIUM OXALATE

Magnesium present (g./100 ml.)	$(NH_4)_2C_2O_4.2H_2O$ (g./100 ml.)	Solubility correction (mg. of Ca/100 ml.)
0·10	7	0·20
0·20	11	0·35
0·30	15	0·60
0·40	20	1·4
0·50	25	2·0

The determinations are completed either by ignition of the precipitate to calcium carbonate at 475 to 500° C., or by permanganate titration. L. A. D.

Solubility of Magnesium Ammonium Phosphate Hexahydrate. R. F. Uncles and G. B. L. Smith (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 699–702)—A systematic investigation of the solubility of magnesium ammonium phosphate hexahydrate in a range of salt solutions is reported. Added salts generally cause a considerable increase in solubility, ammonium salts having a greater effect than equivalent amounts of sodium or potassium salts. Molybdate and oxalate ions have a very great effect, probably by the formation of complex ions. Calcium and barium ions also show exceptional behaviour by increasing solubility. Relatively small quantities of aqueous ammonia reduce the solubility of the compound in every salt solution examined, and with increasing additions of aqueous ammonia the solubility becomes negligible. The solutions commonly employed in the gravimetric determination of magnesium as phosphate by Epperson's method (*J. Amer. Chem. Soc.*, 1928, **50**, 321; ANALYST, 1928, **53**, 239) are such that the solubility is insignificant. The amount of dissolved magnesium was determined by the titan yellow absorptiometric method. The use of hydroxylamine hydrochloride, as suggested by Gillam (*Ind. Eng. Chem.*, *Anal. Ed.*, 1941, **13**, 499), to reduce the rate of fading of the colour, has been found effective.

L. A. D.

Analysis of External Deposits from Boilers. Part I. General Remarks on the Nature of Boiler Deposits and their Examination. H. E. Crossley and A. H. Edwards (*J. Soc. Chem. Ind.*, 1946, **65**, 251–254). **Part II. Method for the Analysis of Deposits Rich in Phosphates. A. H. Edwards** (*Ibid.*, 254–256). **Part III. Method for the Analysis of the Water - Soluble Fraction of the Deposits. A. H. Edwards and D. Flint** (*Ibid.*, 256–257)—The methods described are those used by the British Coal Utilisation Research Association.

Part I—(*a*) *Collection of sample*—Record all relevant details of the samples; *e.g.*, position in the boiler, boiler operation conditions, whether the sample is from exposed or sheltered parts, the proximity to soot-blowers, local gas velocities, the nature of the fuel, etc. Note also the general character of the deposit *in situ*. (*b*) *Nature of the deposits*—The term comprises deposits found on all external surfaces of the boiler from the combustion chamber to the chimney, and the samples are conveniently divided into two groups. *Fly-ash material* consists of solid matter carried mechanically from the fuel bed, and is largely composed of fused or sintered coal ash, although in some parts of the boiler coke or oxidised coal may be present. The amounts of ferric oxide, alumina, titania, lime, magnesia, sodium and potassium oxides, silica, sulphur trioxide, and phosphorus pentoxide total at least 99 per cent., and the sum of the sodium and potassium oxides and phosphorus pentoxide is less than 7 per cent. The *secondary deposit* consists of condensed substances mixed with some fly-ash. The condensed substances usually consist mainly of sulphates and sometimes phosphates of sodium, potassium, iron, and aluminium, together with free silica. There may be present more than 0·5 per cent. each of many elements occurring as traces in the fuel, *e.g.*, boron, copper, mercury, zinc, lead, tin, germanium, gallium, and possibly arsenic. Coal, coke, free acids, and moisture may also be present. Some of the effects of the composition of the sample on the course of the analysis are discussed.

Part II—PROCEDURE—Ignite 2·5 g. of the sample at 800° C. in a stream of pure oxygen, and determine the moisture by absorption in anhydrone, the carbon dioxide by absorption in soda-asbestos, and the sulphur trioxide by absorption on silver gauze at 800° C. Weigh the silver, wash with boiling distilled water, dry by means of alcohol and ether, ignite to dull redness, and reweigh to determine the loss of silver sulphate.

Grind the ignited original material and digest 1 g. with 6 ml. of concentrated hydrochloric acid. Dilute with 20 ml. of hot water, filter, burn the paper, fuse the residue with 3 g. of sodium carbonate, dissolve the fused mass in 10 ml. of concentrated hydrochloric acid and 2 to 3 ml. of 20-volume hydrogen peroxide, and add to the acid extract. Evaporate to dryness, and bake at 115° C. for 2 hr., extract with 5 ml. of concentrated hydrochloric acid and 50 ml. of water and filter off the silica, repeating the evaporation and baking as usual. Ignite the precipitate, treat with hydrofluoric acid, fuse the residue with 0·5 g. of sodium carbonate, add the hydrochloric acid solution of the fused mass to the main filtrate, and dilute the whole to 500 ml.

Sulphate—Dilute 100 ml. of the solution to 300 ml., add 1 ml. of concentrated hydrochloric acid, and 5 ml. of bromine water, and boil to remove excess of bromine. Precipitate barium sulphate by the usual method.

Phosphate—If less than 6 per cent. is present, use the method described in Part III. Otherwise, dilute 100 ml. of the silica-free solution to 250 ml., and add 10 ml. of concentrated nitric acid and 5 g. of ammonium nitrate. Warm to 75° C. and add 100 ml. of ammonium molybdate reagent heated to 40° C. Leave for 0·5 hr. at 50° C., and overnight at room temperature. Filter, leaving most of the precipitate in the beaker, and wash the precipitate and paper with 5 per cent. ammonium nitrate solution. Dissolve the precipitate in ammonia solution (1 + 2), and wash the paper thoroughly with water, keeping the total volume of solution below 200 ml. Add hydrochloric acid until a precipitate appears, and clear the solution by means of 2 or 3 drops of diluted ammonia solution (1 + 1). Add 50 ml. of magnesia mixture, heat nearly to the boiling point, add phenolphthalein and then dilute ammonia solution until the liquid is pink. Cool, add one-fifth of the solution's volume of concentrated ammonia solution and leave for at least 4 hr. Filter, and wash with diluted ammonia solution (1 + 20). Dissolve the precipitate in hydrochloric acid and reprecipitate.

Ignite the precipitate finally at 1000 to 1050° C. and weigh as $Mg_2P_2O_7$.

(*Reagents*—*Ammonium molybdate*—Dissolve 100 g. of pure molybdic acid in 80 ml. of concentrated ammonia solution and 400 ml. of water. Pour the solution slowly into a mixture of 40 ml. of concentrated nitric acid and 600 ml. of water, the mixture being continuously rotated during the process. Add a solution of 5 mg. of sodium ammonium phosphate crystals, heat the mixture to 65° C., shake frequently, and allow to cool overnight. Filter into a dark bottle. Do not keep for more than 2 months.

Magnesia mixture—Dissolve 55 g. of magnesium chloride, $MgCl_2.6H_2O$, and 105 g. of ammonium chloride in water, add a little hydrochloric acid, and dilute to 1 litre.)

Mixed oxides—To 250 ml. of the 500 ml. of solution from the silica removal add enough (usually 15 to 20 ml.) 0·2 *N* ferric iron solution (19·606 g. of ferrous ammonium sulphate dissolved in water and 10 ml. of concentrated sulphuric acid, oxidised by boiling with nitric acid, and diluted to 250 ml.) for precipitation of the phosphate. Add a few drops of nitric acid, heat nearly to boiling and neutralise (to methyl red or rosolic acid) with diluted ammonia solution (1+1). Boil, filter, and wash with cold, 5 per cent. ammonium nitrate solution. Dissolve the precipitate in 7 ml. of concentrated hydrochloric acid and reprecipitate. Filter, wash, ignite, and weigh. Dissolve the precipitate by fusing with 7 g. of potassium pyrosulphate and digesting the melt with dilute sulphuric acid. Evaporate to fuming, dilute, and filter to remove silica, which is recovered, ignited, weighed, and treated with hydrofluoric acid.

Iron—Dilute the solution to 250 ml. and take 100 ml. Oxidise with a few drops of 0·1 *N* potassium permanganate, add 3 ml. of 40 per cent. ammonium thiocyanate, and titrate with 0·05 *N* mercurous nitrate until the red colour disappears. Aluminium oxide plus titanium dioxide is calculated by correcting the weight of the original precipitate for its phosphoric acid and iron contents.

Calcium and magnesium are determined in the filtrate from the mixed oxides as oxalate (titration with potassium permanganate solution), and as magnesium ammonium phosphate, respectively. *Alkalis* are determined on a new portion of ignited material by the Lawrence Smith method.

Part III—*Preparation of water-soluble portion*—Weigh 2 g. of sample (—100 mesh) into a weighed, sintered-glass crucible (Pyrex SF. 2C4), and heat at 105° C. for 2 hr., and for periods of 1 hr. until consecutive weighings agree to within 0·05 per cent. Suspend the crucible by a fine wire in the neck of a 500-ml., round-bottomed bolthead flask, put 20 ml. of water in the crucible and 120 ml. in the flask, fit a reflux condenser, and boil the water in the flask for 2 hr. at such a rate that the crucible does not fill completely. Drain the filter by suction, wash with hot water, dry, and re-weigh. Dissolve any deposit of hydrolysed salts from the flask with two 2-ml. portions of hydrochloric acid and hot water. Keep the solution separate from the main extract. If the main solution contains suspended matter filter it off, dissolve in hydrochloric acid, and add it to the acid washings of the flask. Dilute the clear filtrate to 500 ml. Determine the "mixed oxides" on the acid extract.

Acidity and chloride—Titrate 50 ml. with 0·04 *N* sodium hydroxide to the end-points of methyl red and phenolphthalein. Determine chloride by Volhard's method in the titrated solution.

Sulphate and alkalis—Dilute 200 ml. to 500 ml., add 1 ml. of concentrated hydrochloric acid and 5 ml. of bromine water and boil out excess of bromine. Precipitate the sulphate by adding barium chloride solution, filter, ignite, and weigh. Evaporate the filtrate to 200 ml. and precipitate magnesium as hydroxide by adding a slight excess of barium hydroxide; filter, and wash. Add 20 ml. of freshly prepared, 10 per cent. ammonium carbonate solution to the boiling filtrate, filter, and wash with hot water. Dissolve the precipitate in hydrochloric acid and reprecipitate. Evaporate the combined filtrates to dryness, and heat the residue to 450° C. on a sand-bath to expel ammonium salts. Extract the residue with water, add 3 drops each of saturated ammonium oxalate solution and barium chloride solution, and leave for 4 hr. Add 1 ml. of concentrated ammonia solution and 1 ml. of ammonium carbonate solution, leave overnight, and filter into a weighed platinum dish. Add 0·5 ml. of hydrochloric acid, evaporate to dryness, heat the dish to 450° C., and weigh. Dissolve the mixed chlorides and determine sodium as sodium zinc uranyl acetate, and the potassium as perchlorate by means of perchloric acid and ethyl acetate.

Silicate—Determine by evaporating 200 ml. of solution to which 5 ml. of hydrochloric acid have been added, adding 5 ml. of sulphuric acid, evaporating to fuming, dissolving the salts in diluted hydrochloric acid, and filtering.

Mixed oxides—Determine as in Part II, omitting the addition of iron solution if the phosphorus pentoxide content of the original material is less than 6 per cent.

Iron, Calcium, and Magnesium—Determine as in Part II.

Phosphate—Take a portion of solution containing about 2 mg. of phosphorus, and use the phosphomolybdate method and volumetric finish of B.S. 1016, 1942. L. A. D.

Absorptiometric Determination of Silicon in Steels. C. H. R. Gentry and L. G. Sherrington (*J. Soc. Chem. Ind.*, 1946, **65**, 90–92)—A silicomolybdate complex is reduced to molybdenum blue by means of ferrous iron in presence of oxalic acid. When the sample contains tungsten, a hydrofluoric acid - nitric acid solution is used.

Method—Dissolve 0·25 g. of the sample by the most suitable of the following methods.

(*a*) Simmer with 70 ml. of 5 per cent. sulphuric acid until the sample is dissolved, boil for 5 min. with 30 ml. of 1·2 per cent. potassium permanganate solution, and add 20-volume hydrogen peroxide drop-wise until the hydrated manganese dioxide dissolves. Boil for 3 min., cool, and dilute accurately to 500 ml.

(*b*) Dissolve in 40 ml. of nitric acid (1+4), dilute to 150 ml., boil for 5 min., cool, and dilute accurately to 500 ml.

(*c*) Dissolve in 40 ml. of mixed acid (200 ml. of hydrochloric acid and 65 ml. of nitric acid diluted to 1 litre), dilute to 200 ml., boil for 5 min., cool, and dilute accurately to 500 ml.

(*d*) Add to the sample in a platinum dish 0·50 g. of ammonium fluoride and 12 ml. of nitric acid (1+2). Warm gently until solution is complete, add 1 g. of boric acid dissolved in 10 ml. of warm water, warm for 5 min., and transfer to a 500-ml. graduated flask. Add 12 ml. of nitric acid (1+2) and dilute to 500 ml. Allow to settle somewhat and filter about 100 ml. through a dry paper.

Pipette two 25-ml. portions (A and B) of solution into dry, 100-ml. conical flasks. Add to A 10 ml. of 2·5 per cent. ammonium molybdate solution, mix, and leave for 5 min. Add 10 ml. of 4 per cent. oxalic acid solution, mix, and add 5 ml. of 6 per cent. ferrous ammonium sulphate solution. Set the Spekker absorptiometer water-to-water 1·00 with 608 filters. Fill a 2-cm. cell with solution and take the drum reading. Add to B in order, mixing after each addition, 10 ml. of the oxalic acid solution, 10 ml. of the ammonium molybdate solution, and 5 ml. of the ferrous ammonium sulphate solution. Take the drum reading on this colour blank. The silicon content of the sample is determined from the difference of the readings. To determine a reagent blank, ferric iron must be present. The determination is therefore made on a solution with ferric sulphate added to correspond to the iron content of the sample, or on a standard steel sample of very low silicon content. In some samples, especially weld deposits, in which silicon occurs as silicate as well as silicide, methods (*a*), (*b*), and (*c*) lead to lower results than the hydrofluoric acid method (*d*), owing, presumably, to the insolubility of quartz and some of the silicates in the acid mixtures employed.

The use of oxalic acid prevents interference by phosphorus, arsenic, and vanadium. The reagent blank is chiefly attributable to silicon in the ammonium fluoride. L. A. D.

Determination of Copper in Cast Iron and Steels with Quinaldic Acid. J. F. Flagg and D. W. Vanas (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 436–438)—The application of the reagent to these analyses has been investigated and a satisfactory modification of the method of Zan'ko and Butenko (*Ber. Inst. physik. Chem., Akad. Wiss. Ukr. S.S.R.*, 1938, 9, 99) evolved. It is somewhat long and no more accurate than other methods in general use. The advantages claimed are that the precipitate is of definite composition, the reagent is stable and water-soluble, and only simple apparatus is used. The reagent is prepared by the method of Hammick (*J. Chem. Soc.*, 1923, **123**, 2882), and is used as a 1 per cent. aqueous solution neutralised to *p*H 7 with sodium hydroxide.

Method—Dissolve 1·493 g. of sample in 15 to 20 ml. of *aqua regia*. Add 10 ml. of sulphuric acid (1+1) and evaporate to fuming. Dissolve the residue in 50 ml. of water and as much sulphuric acid (1+4) as is necessary. Filter on a Whatman No. 40 paper and wash the residue once with water, with 25 ml. of a solution of 10 g. of ammonium tartrate, and then once more with water. Add concentrated ammonia solution to the filtrate and washings until the liquid turns a deep cherry-red colour. Cool, adjust the *p*H to 3·0 ± 0·2 with dilute ammonia solution or with sulphuric acid. Heat to 70° to 80° C., add 5 ml. of quinaldic acid reagent for each milligram of copper, leave on a steam-bath for 0·5 hr., and then allow to cool. Collect the precipitate in a filter crucible and wash once or twice with cold water. Dissolve the precipitate in a few millilitres of hot hydrochloric acid (1+1), and rinse the crucible several times with the hot acid and then with water. Add 2 g. of tartaric acid and 2 ml. of quinaldic acid reagent to the solution and then add ammonia solution (1+4) until the *p*H is about 3. Heat and cool as before, filter on a weighed glass crucible, wash with cold water, dry at 115° to 120° C., and reweigh. One mg. of precipitate is equivalent to 0·01 per cent. of copper in the sample. A preliminary concentration of the copper by internal electrolysis may be employed instead of one precipitation, but this method gave a low result with an 18–8 steel. Dissolve 1 g. of sample in *aqua regia* in a tube about 38 mm. in diameter and 130 mm. long. Add 3 ml. of concentrated sulphuric acid and evaporate to fuming. Cool, add 10 ml. of water, boil, and add 1·2 g. of hydroxylamine hydrochloride in 10 ml. of water to reduce the iron. Add 2 ml. of hydrochloric acid (1+1) and 2 ml. of nitric acid (1+3) and immerse the connected electrodes, a platinum gauze cathode and an anode consisting of about 23 cm. of 99·99 per cent. aluminium wire bent into a spiral or loop. Heat the contents of the cell to 70° to 80° C. until the deposition is complete (30 to 75 min.), remove the electrodes, running water over the cathode, and strip the deposit with concentrated nitric acid. Add 5 g. of ammonium acetate to the copper solution, adjust the *p*H to 3 to 3·5, precipitate the copper with 50 mg. of quinaldic acid, filter, wash, and dry as above.

L. A. D.

Determination of [Free] Acid in the Presence of Aluminium [Salts]. R. P. Graham (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 472–474)—The method suggested by T. J. I. Craig (*J. Soc. Chem. Ind.*, 1911, **30**, 184) and subsequently modified by W. W. Scott (Scott and Furman, "*Standard Methods of Chemical Analysis*," 1939, p. 16), [in which the free acid is titrated with standard alkali hydroxide to a phenolphthalein end-point, after addition of a neutralised solution of potassium fluoride to form with the aluminium salt the difficultly-soluble aluminofluoride, K_3AlF_6, and the potassium salt of the combined acid in the sample, but leaving the free acid unchanged], has now been further modified by delaying the addition of potassium fluoride until 99·5 per cent. of the neutralisation has been effected, instead of having it present before starting the titration.

It is shown that when this new modification is applied to solutions of known composition the low results given by the old technique are prevented, and that the higher results by the modified technique are in accordance with theory. The low results are ascribed to occlusion of free acid by the precipitated potassium aluminofluoride. A minimum molar ratio, $F'/Al^{\cdot\cdot\cdot}$, of not less than 6 is necessary in order to satisfy the stoicheiometrical requirements.

The titrations are carried out in paraffin-lined beakers and with solutions free from carbon dioxide. The point at which to add the potassium fluoride is found by means of pilot titrations. In the first of these, the fluoride is added at the beginning of the titration and the result assumed to represent 98 per cent. of the true titre. From this, the volume of standard solution corresponding to 99·5 per cent. neutralisation is calculated. This calculated volume of standard solution is then run in to a second trial titration before the fluoride is added. If the first volume of standard solution added in this second titration is then found to be less than 99·5 per cent. of that at the end-point, the volume added before the addition of the potassium fluoride is re-calculated, and the titration repeated.

The modified fluoride method has been applied to the determination of aluminium in neutral hydrated alumina.

Procedure—Weigh 0·23 g. of the sample and transfer to a 125-ml. conical flask. Add 50 ml. of standard 0·4 *N* sulphuric acid and boil gently, with additions of water to keep the volume constant, until solution is complete (2 to 3 hours). Concentrate to about 25 ml., cool, and titrate the excess of acid with standard alkali and phenolphthalein by the modified fluoride method, using 15 ml. of 2 *M* neutral potassium fluoride solution.

Tested against neutral, air-dried alumina, prepared by treating aluminium amalgam with distilled water, the volumetric method gave results in close agreement with those obtained by ignition to constant weight.

C. F. P.

Determination of Iron in the Presence of Cobalt. E. A. Brown (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 228–230)—The iron is separated from much of the cobalt by one ammonia precipitation. The thiocyanate colour is then assessed by means of an absorptiometer, the effect of the remaining cobalt being eliminated by the use of two colour filters. The method is suitable for determining 0·07 to 0·5 mg. of iron in the presence of up to 90 mg. of cobalt. An accuracy of ± 3 per cent. is claimed. The colour is developed by adding 5 ml. of 10 per cent. ammonium thiocyanate solution to about 90 ml. of solution, containing up to 0·5 mg. of iron and 5 ml. of concentrated hydrochloric acid, and diluting to 100 ml. The absorptiometric readings are made without delay with filters having maximum transmission at 425 mμ and 525 mμ. An expression may be derived by which the iron content of the solution can be calculated from the two readings if it is assumed that the light absorption of the iron and cobalt components obeys Beer's Law and that the components behave independently of each other. The original paper should be consulted for the derivation of the expression, the determination of the constants in it, and a discussion of its range of usefulness.

L. A. D.

Entrainment of Cobalt and Sulphur in Iron Separations. E. T. Pinkney, R. Dick, and R. S. Young (*J. Amer. Chem. Soc.*, 1946, **68**, 1126–1128)—Separation of iron from cobalt and sulphate ions by sodium phosphate gives almost complete recovery of the cobalt (*cf.* North and Wells, *Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 859) and the sulphate in one precipitation. Separation of iron by aqueous ammonia in presence of ammonium salts gives almost complete recovery (98·9–99·6 per cent.) of sulphate in one precipitation, but cobalt is very strongly entrained in the ferric hydroxide precipitate. The amounts entrained vary widely even under standard conditions, and a large number of re-precipitations is necessary to complete the separation from ferric hydroxide in ammoniacal solution. Separation of iron by means of zinc oxide yields most of the cobalt and sulphate in one precipitation if filtration is made in hot solution, although a second precipitation is required for accurate work, but if filtration is carried out in the cold, retention of cobalt is increased slightly and that of sulphate greatly. A table showing the effect of varying conditions in the zinc oxide precipitation gives lowest cobalt and sulphate recoveries when precipitation was in hot, and filtration in cold, solutions. Partial neutralisation of the acid with sodium carbonate before the addition of zinc oxide decreased recovery of both cobalt and sulphate. Large quantities of zinc oxide do not increase entrainment of these ions. E. B. D.

Determination of Uranium with 8-Hydroxyquinoline. A. Claassen and J. Visser (*Rec. Trav. Chim.*, 1946, **65**, 211–215)—The authors have obtained consistently low results when standardising uranium solutions by the method of Hecht and Reich-Rohrwig (*Monatsh.*, 1929, **53/54**, 596), in which the uranium is precipitated by 8-hydroxyquinoline. The method has been subjected to critical examination, and a modified procedure is now proposed.

The negative errors obtained are due partly to the high solubility (0·7 to 1·0 mg. of uranium per 100 ml.) of the precipitate, $UO_2(C_9H_6NO)_2.C_9H_7NO$, in the hot water used for washing. This error can be avoided by washing with hot, dilute oxine solution, in which the precipitate is practically insoluble. Small amounts of oxine adhering to the precipitate are expelled by heating at 110° C. The *p*H of the solution obtained by the procedure of Hecht and Reich-Rohrwig is poorly defined, the acidity often being high enough to prevent complete precipitation of the uranium. Precipitation is now found to be complete only in the range *p*H 5·0 to 9·0. Although Hecht and Reich-Rohrwig report that uranium oxinate remains constant in weight up to 170° C., it was found that 0·3719 g. of the compound, dried at 105° C., remained unchanged by further drying at temperatures up to 140° C., and then lost weight at 150° to 170° C., until at 180° C. decomposition set in. According to Fleck (ANALYST, 1937, **62**, 378), one molecule of oxine is lost by drying at 200° C., but weighing of the precipitate after heating at this temperature is not recommended as the time of heating is critical.

For use in the investigation, a known weight of standard uranium acetate solution containing about 200 mg. of uranium was made 1 to 2 *N* with respect to sulphuric acid and reduced in a cadmium reductor. Air was bubbled for five minutes through the solution, which was then titrated with standard potassium permanganate, *o*-phenanthroline-ferrous complex being used as indicator. As a check, other weights of the standard solution were heated to dryness in platinum with an excess of aqueous ammonia, and the residue ignited to U_3O_8.

Method—The proposed procedure for the oxinate precipitation is as follows: *Reagent*—Dissolve 4 g. of oxine in 8·5 ml. of concentrated acetic acid by slight heating. Pour the clear solution into 80 ml. of water and dilute to 100 ml.

Procedure—Neutralise the uranium solution by adding dilute aqueous ammonia, until a faint turbidity persists or, if tartrate is present, to *p*H 6. Clear the solution by means of a few drops of 2 *N* hydrochloric acid, add 20 to 25 ml. of 20 per cent. ammonium acetate solution, and heat to boiling. Add, drop by drop while stirring, 0·5 ml. of the 4 per cent. oxine solution for each 10 mg. of uranium present, and 4 to 5 ml. in excess. As soon as the precipitate persists, stir until it becomes crystalline before adding more reagent. Finally add, dropwise with stirring, the same volume of *N* ammonia, in order to obtain a *p*H 5·2 to 5·8, and boil for 2 minutes. Allow to cool to 40° C. Filter through a sintered-glass crucible (Jena G4), and wash with 50 to 100 ml. of hot, 0·04 per cent., aqueous oxine solution. Dry at 110° to 140° C. for one to two hours, and weigh as the compound $UO_2(C_9H_6NO)_2.C_9H_7NO$, which contains 33·84 per cent. of uranium. For the volumetric determination, dissolve the dried precipitate in 2 *N* hydrochloric acid, and titrate with 0·05 to 0·2 *N* potassium bromate-potassium bromide solution in the usual way (R. Berg, "*Die Analytische Verwendung von o-Oxychinolin,*" 2. Aufl. Stuttgart, 1938). The uranium oxinate can be ignited to U_3O_8 without the usual precaution of adding oxalic acid to prevent loss of oxinate by sublimation (*cf.* Kroupa, *Mikrochim. Acta*, 1938, **3**, 306). The dried precipitate is heated in an electric muffle, first at 1000° C., and then at 700° C. for one hour.

Results obtained by the gravimetric or volumetric procedures are 0·2 per cent. low for amounts of uranium greater than 50 mg., and accurate to 0·1 mg. for lesser amounts. When the oxinate is ignited and the uranium weighed as U_3O_8, the results obtained are accurate to within ±0·1 per cent. for up to 400 mg. of uranium. The slightly low results obtained by weighing the oxinate must be due to a small deficiency in the oxine content of the precipitate. Accuracy is unimpaired by the presence of several grams of tartaric acid, and complete separation from magnesium, calcium, strontium, and barium is obtained. B. A.

Determination of Gold with Ascorbic Acid. E. C. Stathis and H. C. Gatos (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 801)—The method is based on the reaction

$$2\ AuCl_3 + 3C_6H_8O_6 \longrightarrow 2Au + 3C_6H_6O_6 + 6HCl.$$

Procedure—Dissolve up to 0·3 g. of gold in *aqua regia* and evaporate to fuming with concentrated hydrochloric acid three times. Dissolve the residue in 3 to 5 ml. of concentrated hydrochloric acid and dilute to 20 ml. with water. Heat the solution to between 80° and 90° C., and add 10 ml. of freshly prepared, 0·4 per cent. ascorbic acid solution; heat for 5 min. Allow to cool, and filter on to a porcelain filter crucible. Wash with diluted hydrochloric acid (1+99) and ignite.

Results show a maximum deviation of 1 in 290, the presence of up to equal amounts of copper being without effect.

Volumetric determination—The excess of ascorbic acid is titrated with iodine according to the scheme $C_6H_8O_6+I_2 \longrightarrow C_6H_6O_6+2HI$.

Procedure—Add to the gold solution, freed from oxidising agents, 2 to 5 ml. of concentrated hydrochloric acid and dilute to 20 ml. Add 10 ml. of ascorbic acid solution, stir for 5 min., add 2 ml. of starch solution, and titrate with 0·1 *N* iodine. Blank determinations must be made. A maximum deviation of 1 in 475 is recorded. M. E. D.

Detection of Ytterbium in Rare-earth Mixtures. G. Beck (*Helv. Chim. Acta*, 1946, **29**, 506–507)—The high reduction potential of bivalent ytterbium, produced by the action of magnesium or, better, sodium amalgam, enables it to reduce the carboxyl groups of certain organic acids to carbonyl groups which are easily detected as coloured derivatives. Oxalic acid is reduced to glyoxalic acid which forms a pink-coloured condensation product with naphthoresorcinol.

Procedure—Treat 1 to 2 ml. of an ice-cooled solution of the sulphates of the rare earths in dilute sulphuric acid with 0·5 ml. of sodium amalgam in a small separating funnel. When reduction is complete, remove the amalgam, and add 3 to 4 drops of boiled-out, saturated oxalic acid solution, to form not more than a slight turbidity. Add a small quantity of naphthoresorcinol and an equal volume of concentrated hydrochloric acid, heat to boiling for 1 to 2 min., cool, and dilute with water. On extraction of the solution with 1 to 2 ml. of ether, a pink coloration indicates the presence of ytterbium.

The reaction is not given by reduced titanium, europium, chromium, molybdenum, zirconium, or tantalum, but vanadium, uranium, niobium, and rhenium give positive results. Oxidising acids interfere, for divalent ytterbium is unstable, even in contact with air. Pyrogallol and sulphuric acid, or tryptophan and sulphuric acid, can be used instead of naphthoresorcinol and hydrochloric acid, and phthalic anhydride can replace oxalic acid. A distinct coloration is obtained with 3 μg. of ytterbium under optimum non-oxidising conditions. The test was negative with cerium earths and yttrium fractions, weakly positive with gadolinium fractions, and strongly positive with erbium preparations. If, after reduction with zinc and hydrochloric acid, the cacothelin test is negative, the reaction is specific for ytterbium. M. E. D.

Agricultural

The Morgan Method of Soil Testing. Part IV. Use of the Spekker Absorptiometer for Estimating Phosphate. J. Tinsley and N. H. Pizer (*J. Soc. Chem. Ind.*, 1946, **65**, 208–211)—Following the work on the direct estimation of potassium, an examination was made of the conditions required for the direct estimation of phosphate by means of the molybdenum-blue reaction, using 1 ml. of soil extract and the measuring and other apparatus found suitable for the potassium test (*Idem, ibid.*, 1945, **64**, 182). The universal extracting solution (U.E.S.) used by Morgan (Tinsley and Pizer, *loc. cit.*, *Connecticut Agr. Expt. Sta.*, 1937, Bull. 392; 1939, Circ. 127) consists of a buffer mixture of sodium acetate and acetic acid having a *p*H of 4·8. Extracts of field soils usually contain from 0 to 10 p.p.m. of phosphorus in the form of phosphate with variable amounts of other ions and small amounts of organic matter. In Morgan's original method. Phosphate is estimated directly on a spot plate by formation of molybdenum blue with sodium molybdate and stannous oxalate, 0·5 ml. of soil extract being used and the volume being increased by addition of reagents to only 0·65 ml., so that the final concentration of phosphate in a small volume of U.E.S. at a final concentration of 0 to 8 p.p.m. of phosphorus, using 13-mm. diameter glass tubes for the formation and measurement of the colour. By contrast, the final concentration of phosphorus in the method of Truog and Meyer (*Ind. Eng. Chem., Anal. Ed.*, 1929, **1**, 136) is of the order 0 to 0·25 p.p.m. and measurement is made in 1-cm. or 4-cm. cells.

Considerable modification of Morgan's process was found necessary, and stannous chloride proved to be a more suitable reagent than stannous oxalate. Colour formation was most intense when the sodium molybdate reagent contained 1·5 *N* hydrochloric acid, but with calcareous soils false results might be obtained, apparently owing to the reduced acidity or to the raised *p*H and not to the presence of calcium. This is important since soil extracts contain variable and often high amounts of calcium. The error may be corrected by addition of sufficient hydrochloric acid to the sodium molybdate reagent. With ordinary U.E.S. and with stannous chloride at a concentration of 0·1 per cent. in 0·1 *N* hydrochloric acid, the intensity of the blue colour depends upon the amounts of sodium molybdate and hydrochloric acid in the molybdate reagent (Woods and Milton, *Ibid.*, 1941, **13**, 760). With calcium-neutralised U.E.S., the sodium molybdate in the reagent must not exceed 1 per cent. With 0·1 per cent. stannous chloride in 0·1 *N* hydrochloric acid and 1 per cent. sodium molybdate reagent, the concentration of the hydrochloric acid in the latter may range from 2·0 to 2·5 *N* for standards in ordinary U.E.S. and should be exactly 2·5 *N* for calcium-neutralised U.E.S. For most soil extracts the best concentration of hydrochloric acid in the sodium molybdate reagent appears to be 2·5 *N*, and this reagent should give accurate results with extracts of acid soils and slightly high results with calcareous soils, a maximum positive error of 5 per cent. being obtained when the acetic acid is fully neutralised. A final *p*H of 0·36 to 0·18 appears to be desirable. Stannous chloride concentration may range from 0·05 to 0·15 per cent. with little effect on colour development, but 0·1 per cent. is preferable with soil extracts of high phosphate content, since at this concentration fading of the colour is inhibited. With 0·1 per cent. stannous chloride reagent and 1 per cent. sodium molybdate in 2·5 *N* hydrochloric acid, colour development reaches a maximum in 2 min. in standards and in most soil extracts and remains constant for 30 min.

If the drum readings on the Spekker absorptiometer are plotted against p.p.m. of phosphorus in standard solutions, the points fall on a straight line over the range 0 to 8 p.p.m., and the curves for standards in ordinary U.E.S. and calcium-neutralised U.E.S. are almost coincident.

Method—The following method was tentatively adopted for routine estimation of phosphate in Morgan soil extracts, the molybdenum-blue colour being developed and measured in 13-mm. diameter glass tubes as described for the determination of potassium. To prepare the stock phosphate solution, containing 100 p.p.m. of phosphorus, dissolve 0·57435 g. of Sörenson's salt ($Na_2HPO_4.2H_2O$) in U.E.S. and dilute to 1 litre. Thymol may be added as preservative. To prepare standard solutions, dilute the stock solution with U.E.S. The sodium molybdate reagent contains 1 per cent. of sodium molybdate ($Na_2MoO_4.2H_2O$) in exactly 2·5 *N* hydrochloric acid, and is stable for 2 months in a stoppered, brown-glass bottle. To prepare the stock stannous chloride solution dissolve 2·5 g. of stannous chloride ($SnCl_2.2H_2O$) in enough hydrochloric acid to give 5 *N* acid after dilution to 50 ml. with water; it is stable for 2 weeks when stored in a refrigerator. The working stannous chloride solution is a 50-fold dilution of the stock solution. The apparatus is as previously described for the estimation of potassium (Tinsley and Pizer, *loc. cit.*)

Procedure—Fix a wooden block holding the requisite number of tubes firmly on the platform of the shaker, measure 1 ml. of phosphorus standard or soil extract into each tube, pour 1 ml. of sodium molybdate reagent down the side of each tube, and shake the mixture for 15 sec. Add 0·5 ml. of stannous chloride reagent to each tube shaking immediately by hand to mix and then in the shaker for 10 sec. After the tubes have stood for 10 min., place them in turn in the special brass holder on the Spekker instrument and measure the intensity of the colour. A 10-ml. pipette, subdivided to 0·1 ml. and having two extra 1-ml. marks at the upper end to provide molybdate reagent for 10 extracts and 1 standard and to allow rejection of the first millilitre drawn in, is recommended for measuring the molybdate reagent and, for the same reason, a 5-ml. pipette subdivided to 0·01 ml. and having two extra 0·5 ml. marks at the upper end is recommended for measuring the stannous chloride reagent. Warm the lamp of the instrument by switching on for 30 sec. and off for 15 sec., and repeating this four times. Use No. 1 red filters and place a tube containing water in the special brass holder against the lens holder on the right. Set the diaphragm at 1·5 on the drum with maximum sensitivity control and reduce the deflexion to zero. Construct the calibration curve as for potassium with 90 selected tubes to carry out 10 independent measurements with each of nine standards (0·5, 1·0, 2·0, . . . 8·0 p.p.m.). Multiply the mean drum reading for each set of standards by 100 and deduct this figure from 150 (the position of zero setting). Since the scale is logarithmic, this value is directly related to the phosphorus content. The calibration curve is a straight line from 1 to 8 p.p.m., but changes slope somewhat from 0 to 1 p.p.m. The curve obtained when 1-cm. optical glass cells are used is a straight line from 0 to 8 p.p.m. In routine determinations ten soil extracts are measured at a time, and one standard, usually in the range 5 to 6 p.p.m., is included as a check on the reagents and instrument. Day-to-day agreement with the curve is good and re-calibration is not necessary for months unless changes occur in the photocells, or unless the lamp is changed.

Temperature changes over the range 5° to 30° C. have slight effects on colour development, but these are not important in routine work. The following ions when included in the standards had no measurable effect on colour development or stability even in amounts greater than those occuring in soil extracts: magnesium, manganese, metasilicate, and iron, but a final concentration of 40 p.p.m. of iron markedly reduced the intensity of the blue colour in standards containing more than 1 p.p.m. of phosphorus. A. O J.

Bacteriological

Procedure for the Characterisation of the Acetic Acid Bacteria. Parts I and II. J. Tošić and T. K. Walker (*J. Soc. Chem. Ind.*, 1946, **65**, 104–107, and 180–184)—In Part I consideration is given to the precautions that must be adopted in standardising conditions for the examination of cultures of *Acetobacter* species in order that comparisons may be obtained for diagnostic purposes. Bacteriologists who first described species of

Acetobacter studied their characteristics in a wide variety of media and early communications are notable for the omission of details that are essential to proper comparison. Standard media and technique are first described in Part I, and the behaviour of ten authentic cultures of named species of *Acetobacter* (obtained from the National Collection of Type Cultures at the Lister Institute) when submitted to the standardised examination is recorded in Part II.

The standard media include Standard Malt Wort (S.M.W.); S.M.W. containing 2 per cent., 1·5 per cent., and 0·5 per cent. of agar, the latter being employed for testing motility; S.M.W. containing gelatin; nutrient broth; broth agar and broth gelatin; yeast water media including carbohydrate, yeast water and corresponding agar; and glucose phosphate broth for the Voges-Proskauer reaction. Standard inoculation procedures are described in detail. Stock cultures are inoculated into S.M.W. and incubated at optimum temperatures for 72 hr., after which a second subculture is made, incubated for 48 hr., and used to inoculate a third subculture which, after 24 hr. incubation, is used to inoculate the test media.

Differentiation is based upon morphological, cultural, biochemical, and physiological characteristics, which are described in detail. In Part II, it is shown that colonial appearance on S.M.W. agar serves to distinguish *A. ascendens* and *A. capsulatum* from all other species, and that the last-named alone forms spreading colonies; that streak cultures also distinguish *A. capsulatum* and the group *A. acetigenum*, *A. pasteurianum*, and *A. xylinum*; the last three also give characteristic growth in yeast water. Growth in S.M.W. containing varying concentrations of ethyl alcohol sharply distinguishes *A. gluconicum*, which is intolerant of 6 per cent., from *A. acetosum* and *A. ascendens*, which tolerate 10 per cent., the other species falling between these two extremes. Acid formation in yeast water containing various carbohydrates serves to distinguish quite a number of species, *e.g.*, only two, *A. capsulatum* and *A. suboxidans*, produce acid from maltose; only two, *A. aceti* and *A. acetigenum*, produce no acid from arabinose; only one, *A. xylinum*, fails to form acid in ethylene glycol and three are distinguished by their ability to oxidise acetic acid; these are *A. acetigenum*, *A. ascendens*, and *A. aceti*. D. R. W.

Physical Methods, Apparatus, etc.

Effect of Sieve Loading on the Results of Sieve Analysis of Natural Sands. F. A. Shergold (*J. Soc. Chem. Ind.*, 1946, **65**, 245–249)—The amount of material put on to a sieve can seriously affect the accuracy of a sieve analysis, and samples should generally be as small as possible. The loading effect of a material on a particular sieve is largely determined by the proportion of "near-mesh" particles, *i.e.*, particles which are just small enough to pass through the mesh apertures, and is influenced by particle shape. Flaky material is slightly more difficult to sieve than a sample composed of equi-dimensional particles, and elongated particles are very difficult to sieve. It is much better to reduce the weight of the sample on a given sieve than to sieve for a longer time. If the sieves are not overloaded the customary 9-min. shaking need not be exceeded unless high accuracy is required. Theoretically, the effective load on a sieve depends on the number of "near-mesh" particles per aperture, but practical tests suggest that larger mesh sieves overload more easily than finer sieves at a given amplitude of the sieve-shaking motion. The particle-size distribution within the sample has an important effect on the loading of sieves, and it is necessary to assume that a high proportion of difficult particles are present when determining the sample weight. With a natural sand the sample weight should be such that, after sieving, not more than the following amounts remain on the respective sieves:

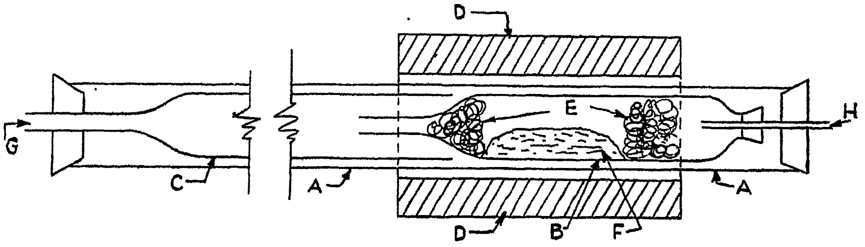
B.S. Sieve No.	7	14	25	52	100	200
Weight retained (g.)	150	100	70	50	35	25

Values for intermediate sizes of sieves may be interpolated, but if the intermediate sieves are used in addition to those listed, all the weights should be halved as the proportion of "near-mesh" particles will be doubled. For crushed sands or rocks, smaller samples should be taken because of the less favourable particle shape. Usually, with natural sand, the original sample for the series of sieves listed is 100 to 150 g. for coarse sand and 40 to 60 g. for fine sand.

Recommendations based on the work described in this paper are incorporated in the revised British Standard for Concrete Aggregates (B.S. 882:1944, Appendix B). L. A. D.

Simple Sublimation Apparatus. J. Pitha (*J. Chem. Educ.*, 1946, **23**, 403)—The apparatus described is easy to construct and operate, and gives pure sublimates in good yield. The tube, A, is a 4-ft. length of 55 mm., O.D., Pyrex glass. The charge tube, B, is made by drawing out 45-mm. O.D., Pyrex tubing to a diameter of about 20 mm. at one end and tooling for a No. 8 rubber stopper at the other, the total length of the tube being about 2 ft. The collector tube, C, consists of about 20 in. of 45-mm. Pyrex tube sealed to a 12-in. length of 20 mm. tube at one end. The furnace, D, is wound on 18 in. of 2·5-in. leader pipe, and is covered with asbestos and rock-wool insulation, its construction allowing regulation of the temperature by a variable transformer.

Procedure—Place the tube, A, in the furnace and heat to the desired temperature. Introduce the collecting tube into A and hold it in place by a rubber bung between the outer tube and the drawn-out end, then connect with a vacuum line or aspirator. Charge B with E, the material to be sublimed, glass wool plugs, E, being used to prevent spreading. Set the charged tube in place butting against the end of C, and adjust a gas delivery tube, H, through the stoppers in the ends of B and A. Gases may be passed through this tube, if desired.



The apparatus has been used to give good yields of highly-pure sublimates of arsenious oxide and phosphorus pentoxide. Crystalline selenium dioxide was obtained by using a stream of oxygen containing nitric oxide, a charge of 350 g. being sublimed in 90 min. The materials of which the apparatus is made must be modified for use at temperatures above 480° C., quartz or Vycor then being suitable.

M. E. D.

Reviews

FORENSIC CHEMISTRY. By HENRY T. F. RHODES, Dip.Inst.C. (Lyon). Pp. vii + 164. London: Chapman & Hall, Ltd., 1946. Price 15s. net.

In this book the author's general plan is to give a brief survey of the chemistry of each section of his subject, together with a collection of the appropriate, well-known (mostly qualitative micro-) analytical methods. From the purely analytical point of view, therefore, the book is likely to be of more use to the student of elementary forensic chemistry than to the expert analyst, who will find very little that is not already familiar to him. However, the latter will, as occasion arises, do well to consult it for much useful and interesting information of a non-analytical type, although the chemical treatment is, on the whole, elementary.

The ground covered is wide, as is evident from the chapter headings, *viz.*, direct and indirect identification of the person, stains, firearms and explosives, and the chemical examination of questioned documents, counterfeit money and toxic agents. The only notable omission (and one very difficult to account for) is textile fibres and fabrics, which receive brief treatment in 14 lines—and this despite the great amount and forensic importance of the knowledge now available concerning the chemical identification of fibres.

The title of the book has been followed strictly, perhaps too strictly in certain respects; thus, the use of ultra-violet light is very often much more rapid and convenient than chemical methods in dealing with stains, questioned documents, faded writing and sympathetic inks, but this fact may not be apparent to many of the readers to whom the book is addressed. With this (*i.e.*, the student) type of reader still in mind, the reviewer considers that the book would be improved by the inclusion of more working details of the analytical methods, and of illustrations; the absence of the latter is felt particularly in the sections dealing with fibres and with microchemical tests involving the formation of crystals of specific shapes.

There is much in the section on paper that the reviewer cannot agree with. In most cases the tests described will be less efficient than the human eye in dating a piece of paper or identifying it with another sample; the staining tests described are quite ineffective in distinguishing the majority of paper-making fibres, and need to be supplemented by microscopical examinations of structures; the sulphate content of paper is not a reliable indication of the presence of sulphite pulp (p. 101); artificial watermarks are by no means invariably removed by ether (p. 127); paper surfaces are not sized with rosin to produce smoothness (p. 102); organic pigments are confused with organic dyes on p. 104; good grade adhesives give neither a starch reaction with iodine nor do they appreciably reduce Fehling's solution (p. 123).

There is also a sprinkling of spelling errors, and the well-known test for nitrogen is referred to as the "black ring test" (p. 70). The style is clear, and the book is well documented with references to the sources from which information is drawn and with titles for further reading—a useful feature. It is indeed unfortunate that the defects noted above have to be recorded, but they can be rectified in the future edition that is bound to be called for.

The book is well produced, though when one bears in mind its size, the absence of illustrations and the fact that it is a second edition, the price must definitely be regarded as on the high side. JULIUS GRANT

PHYSICAL METHODS GROUP

THE next meeting of the Physical Methods Group will be held in the Chemistry Lecture Theatre at King's College, Newcastle-upon-Tyne, at 6 p.m. on Friday, May 2nd, 1947. The following short papers on Physical Methods of Gas Analysis will be read and discussed:

"Gas Analysis at Low Pressures." By C. E. Ransley, M.Sc., Ph.D.

"The Analysis of Hydrocarbon Gases by Low Temperature Distillation." By J. H. D. Hooper, B.Sc., A.R.I.C.

"A New Apparatus for Gas Analysis by the Soap Film Method." By W. J. Gooderham, B.Sc., A.R.C.S., F.R.I.C., M.Inst.Gas E. Mr. Gooderham will demonstrate his apparatus.

Facilities will be available for a limited number of Members and visitors to take tea at King's College at 5 p.m. Members wishing to do this must advise Dr. J. O. Harris, Chemistry Department, King's College, Newcastle-upon-Tyne, 1, before Friday, April 25th.

POLAROGRAPHIC DISCUSSION PANEL

THE first ordinary meeting of the Polarographic Discussion Panel will be held at the Imperial College of Science and Technology, Imperial Institute Road, South Kensington, London, S.W.7, at 3 p.m. on Friday, April 25th, 1947, when a general discussion on Polarographic Analysis will be opened by Dr. W. Cule Davies, Dr. G. Jessop, and Dr. D. R. Roberts. Full details will be circulated to Members of the Polarographic Discussion Panel. Members of the Physical Methods Group who wish to become members of the Polarographic Discussion Panel are asked to inform Mr. J. T. Stock, the Honorary Secretary of the Panel, at the Chemistry Department, L.C.C. Norwood Technical Institute, Knight's Hill, London, S.E.27.

APRIL, 1947 Vol. 72, No. 853

7 JUN 1947

THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

THE Annual General Meeting of the Society was held at 3.30 p.m. on Friday, March 7th, 1947, in the meeting room of the Royal Society, Burlington House, London, W.1. The chair was taken by the President, Dr. G. W. Monier-Williams. The Financial Statement for 1946 was presented by the Hon. Treasurer and approved and the Auditors for 1947 were appointed. The Report of the Council for the year ending March, 1947 (see pp. 131–137) was presented by the Hon. Secretary and adopted. The following were elected Officers and Council for the coming year.

President—Lewis Eynon, B.Sc., F.R.I.C.

Past Presidents serving on the Council—F. W. F. Arnaud, Bernard Dyer, E. B. Hughes, G. Roche Lynch, S. Ernest Melling, and G. W. Monier-Williams.

Vice-Presidents—R. C. Chirnside, D. W. Kent-Jones, (Mrs.) J. W. Matthews and, *ex officio*, C. H. Manley (Chairman, North of England Section), and H. Dryerre (Chairman, Scottish Section).

Hon. Treasurer—G. Taylor.

Hon. Secretary—K. A. Williams.

Other Members of Council—C. A. Adams, R. Belcher, H. E. Cox, (Miss) I. H. Hadfield, J. H. Hamence, J. Haslam, A. D. Mitchell, J. R. Nicholls, Norman Strafford, R. W. Sutton, H. N. Wilson, E. C. Wood and, *ex officio*, Arnold Lees (Hon. Secretary, North of England Section), and R. S. Watson (Hon. Secretary, Scottish Section).

After the business outlined above had been completed the meeting was opened to visitors, and the retiring President, Dr. G. W. Monier-Williams, O.B.E., M.C., M.A., F.R.I.C., delivered his Presidential Address, in which he made reference to some outstanding features in the affairs of the Society during his tenure of the presidency, and then proceeded to a consideration of certain aspects of food law administration with particular reference to the control of the use of preservatives and the presence of injurious substances in foods (see pp. 137–142).

NEW MEMBERS

Richard Ralph Appleby, B.Sc. (Lond.); Geoffrey Browne, B.Sc.(Lond.), F.R.I.C.; Stanley James Bush, A.R.I.C.; Terence John Cahill, F.R.I.C.; (Miss) Eileen Mary Chatt, B.Sc. (Lond.), F.R.I.C.; Thomas Morton Clark, B.Sc. (Lond.); Harry Kenneth Dean, B.Sc., Ph.D. (Liv.), F.R.I.C.; John Arthur Eggleston, B.Sc. (Lond.), A.R.I.C.; Charles Arthur Hallas, B.Sc.(Lond.), F.R.I.C.; Wynford Price Jones, A.R.I.C., Ph.C.; Ralph William Latter, B.Sc. (Lond.), A.R.I.C.; George Frederick Longman, B.Sc. (Lond.), F.R.I.C.; Thomas Barton Mann; John Vernon Morris, B.Sc. (Lond.), A.R.I.C.; Geoffrey Padget, Assoc.Met. (Sheff.); Arthur Edward Pickford, A.R.I.C.; Joseph Brian Rickson, B.Sc. (Lond.), A.R.I.C.; Albert Arthur Smales, B.Sc. (Lond.), A.R.I.C.; William Stross, M.D. (Prague); Cyril Gordon Sumner, M.Sc., Ph.D. (Manc.), A.R.I.C.; Leslie Stuart Theobald, M.Sc. (Lond.), A.R.C.S., F.R.I.C.; Walter Frederick Wilkinson, M.P.S.

Anniversary Dinner

IN the evening of the day of the Annual General Meeting the Society held an Anniversary Dinner, the first since 1938, at the Dorchester Hotel, Park Lane, London, W.1, to celebrate its seventy-third anniversary.

The members and guests, who numbered 110, were received by the President, Dr. G. W. Monier-Williams and Mrs. Monier-Williams The President afterwards took the chair at the dinner.

The guests of the Society and of the President were: Professor Sir Robert Robinson, M.A., D.Sc., LL.D., F.R.I.C., President of the Royal Society; Sir Arthur S. MacNalty, K.C.B., M.D., F.R.C.P., K.H.P., formerly Chief Medical Officer, Ministry of Health; P. N. R. Butcher, Esq., Assistant Secretary, Ministry of Health; Dr. J. Alison Glover, O.B.E., F.R.C.P., formerly

Linlithgow Library.
Imperial Agricultural Research Institute.

Senior Medical Officer, Ministry of Education; Dr. G. M. Bennett, M.A., Sc.D., F.R.I.C., F.R.S., Government Chemist; Professor Alexander Findlay, M.A., Ph.D., D.Sc., LL.D., Past President of the Royal Institute of Chemistry; Dr. L. H. Lampitt, F.R.I.C., M.I.Chem.E., President of the Society of Chemical Industry; R. O. Hall, Esq., and Mrs. Hall.

After the loyal toasts, proposed by the President, had been duly honoured, Professor FINDLAY proposed the health of the Society. Beginning with an outline of the early history of the Society, founded by a small number of Public Analysts who were deeply interested in the health and welfare of the community and anxious that the administration of the then newly enacted Food and Drugs Adulteration Acts should not fall into the hands of incompetent or unscrupulous practitioners, Professor Findlay traced its development, during the past seventy-three years, to its present position as one of the leading Societies for the study and propagation of analytical chemistry. This progress had been made in close collaboration and co-operation with the Royal Institute of Chemistry and had been greatly accelerated and expanded by the inclusion, in the Society, of analytical chemists other than Public Analysts and, more recently, by the formation of groups within the Society, for the special study of microchemical, physical, and biological methods of analysis. These ever-growing activities, coupled with the increased circulation and prestige of THE ANALYST, which had now become the most important journal of analytical chemistry in this country and one of the most important in the world, had made it necessary for the members to realise that the Society had now become the guardian of analytical chemistry. He expressed regret that there should still be no Chair of analytical chemistry in Great Britain, although his own University, Aberdeen, had recently appointed a member of the Society as a lecturer in that subject. He concluded by recalling older, more intimate annual dinner parties which were often enlivened by musical contributions by members. He particularly regretted that Dr. Bernard Dyer, the sole remaining original member of the Society, was unable to be present, though he was quite well and with us in spirit.

The PRESIDENT, in replying to the toast, thanked Professor Findlay, on behalf of the Society, for the kind way in which he had referred to its activities. He said that although our Society was not the oldest of the publishing societies (that honour fell to the Chemical Society, which was holding its delayed centenary celebrations this year) it had undergone a more significant development than any of the others. Starting as a very small group of enthusiastic men, for the most part Public Analysts, it had now become the central society for all analytical chemists, but despite its great increase in membership and diversity of interests, it had retained throughout its existence a close association with Public Analysts, whose great services to our Society, throughout its past and at the present time, was recognised by all our members. The proposer of the toast had mentioned the remarkable diversity of analytical methods that had been evolved in recent years. In these activities the Chairmen and Committees of the new subject Groups were to be congratulated upon the valuable work that they were doing. The success of the Society as a whole was proved by the demand for THE ANALYST, particularly by increased sales to non-members. For many years THE ANALYST had been well served by Dr. Ainsworth Mitchell. It had now a strong Publication Committee and an augmented editorial staff, that looked like having some hard work ahead. The Publication Committee prided itself on producing a distinctive type of informative abstract. The difficulty of covering the whole of the published analytical work was almost insuperable; that used for abstracting was selected with care and discrimination from the original papers that appeared to be of the greatest practical value. In conclusion, he thanked Professor Findlay for the interest that he had always shown in analytical chemistry as an educational subject and for the able and effective way in which he had furthered the interests of our Society.

Mr. A. L. BACHARACH, in proposing the health of the kindred Societies, welcomed Sir Robert Robinson, President of the Royal Society, who in proposing a similar toast, some years ago, had recognised the Society of Public Analysts as one of the Learned Societies of this country. He wished Sir Robert a safe journey and success in his forthcoming visit to America. He regretted that the President of the Chemical Society had found it impossible to be present. On behalf of the Society of Public Analysts he wished the Chemical Society, and its President, a successful celebration of its centenary, which should have been held six years ago; for not only was that Society the oldest, it was also the most important Chemical Society in the world. He regretted also the absence of the President of the Royal Institute of Chemistry, Dr. G. Roche Lynch, who was on a visit to Ireland, but we had the pleasure of the presence of his predecessor in that office, Professor Alexander Findlay. In Dr. Lampitt we had with us the President of the Society to which we were the most closely linked by our activities, the Society of Chemical Industry. Dr. Lampitt was concerned with food not only as the founder of that Society's Food Group, but also as its first Chairman and the instigator of the annual joint meeting held by the Group and our Society. For his part, he saw no reason why the practice of joint meetings should not be extended to other Groups of the Society of Chemical Industry.

Dr. LAMPITT, replying for the Kindred Societies, said that they were kindred in spirit—the spirit of science at its best and free from the baser uses to which science could be put. But he trusted that while retaining the spirit of kinship and co-operation, they would never amalgamate, for only through competition could they remain virile.

The health of the Guests, coupled with the name of Dr. G. M. Bennett, Government Chemist, was proposed by Dr. H. E. Cox, who likened his Toasts to a constellation of stars, and in a felicitous speech placed them in their several orders of magnitude and traced their various orbits.

Dr. G. M. BENNETT, replying for the guests, thanked the Society for an enjoyable evening as well as for the opportunity of acknowledging a debt owed to the Society by all chemists. He said that THE ANALYST had a very high standard and that he had been much impressed by its prompt publication of papers; in this respect it compared favourably with journals of other societies. He mentioned the Society's entry into the Chemical Council and said that this would be to their mutual advantage. He spoke of the cordial relationship that exists between his Department and the Society, also of the pleasure that he took in helping its members in any way possible.

Sir ROBERT ROBINSON made some noteworthy observations on the importance of analytical chemistry and said that a committee had recently been formed to discuss the teaching of that branch of science in universities. He urged the need for a University Chair in analytical chemistry and asked for the active assistance of all chemists in securing that object.

In proposing the health of the President Elect, Mr. Lewis Eynon, the PRESIDENT said that Mr. Eynon had served the Society as Honorary Secretary for the past eleven years, but that he, personally, had known him for many more years than that, having met him for the first time in a trench in Northern France. He assured the members that a better man could not have been chosen for the office of President.

Mr. EYNON replied in a short and appreciative speech.

Finally the PRESIDENT proposed that a message be sent to Dr. Bernard Dyer conveying the Society's good wishes and lively appreciation of his work in its interest. The proposal was received with acclamation.

Annual Report of Council: March, 1947

THE roll of the Society numbers 1345, an increase of 75 over the membership a year ago.

The Council regrets to have to record the death of the following members:

H. G. Battye
J. J. Blackie
G. E. Boizot
A. Bruce
J. L. Buchanan
W. J. A. Butterfield
W. R. Dracass
G. W. Edwards
H. Firth
J. A. Foster
P. F. Frankland
C. D. Garbutt
A. D. Heywood
E. Hinks
P. H. Jones
J. F. Liverseege
T. McGrath
R. S. Morrell
M. Nierenstein
F. E. Needs
W. H. Roberts
K. H. Vakil
G. H. Warburton
S. Watkinson

Battye, who died in his 58th year, was elected a member of the Society in 1940. He studied at the Central Technical College and Leeds University, and after being engaged in private practice for some years he went to Canada. In 1941 he became an Inspector of Explosives and was subsequently a chemist in the Department of Mines, Ottawa.

Boizot, who died at the age of 33, received his scientific training at Battersea Polytechnic and obtained the B.Sc. degree with first class honours. He was subsequently employed by May & Baker, Ltd., and in 1931 became Food Analyst to United Dairies, Ltd. In 1938 he was appointed Assistant Government Analyst at Singapore. He died in 1943 whilst a prisoner of war.

Bruce, who died in his 69th year, was elected a member of the Society in 1925. He studied at Edinburgh University and obtained the B.Sc. degree in 1900. After a short period of service at the Royal Arsenal, Woolwich, he went to Ceylon and engaged in consulting and analytical practice. He published a number of papers and became a Fellow of the Royal Society of Edinburgh.

Buchanan, who died at the age of 73, became a member of the Society in 1923. He worked for some years in the laboratory of Tatlock, Readman and Thomson, City Analysts, Glasgow. In 1894 he joined Messrs. Lever Bros. at Port Sunlight, became a Managing Director in 1911 and, in 1922, Chairman of the Associated Company, John Knight, Ltd.

Butterfield, who died in his 80th year, had been a member of the Society for 43 years. He received his scientific training at Oxford, obtaining the B.A. degree with honours in chemistry and subsequently M.A. In 1891 he became chemist to the Gas Light and Coke Co., Beckton, and afterwards a Gas Referee under the Board of Trade, and in 1938 Advisor to the Board of Trade under the Gas Undertakings Act, 1934. He was also engaged in consulting practice. He served for two periods as an Ordinary Member of the Council of the Society and, in 1920–21, as Vice-President.

Dracass, who died in his 37th year, was elected a member of the Society in 1933. He studied at King's College, London, where he graduated B.Sc. in 1930, and at the Sir John Cass Technical Institute, obtaining the M.Sc. degree in 1935. He worked for some years in the laboratory of the late Mr. E. Hinks, then with Messrs. Burroughs, Wellcome & Co., and in 1944 he became Senior Analyst to Dr. H. E. Cox until, shortly before his death, he became employed by Allied Supplies, Ltd.

Edwards, who died in his 26th year, was elected a member of the Society in 1944. He was trained at the Manchester College of Technology and was for 8 years an assistant analyst to Messrs. Melling & Ardern. In 1943 he was appointed as a chemist to the War Department.

Firth, who died in his 46th year, became a member of the Society in 1926. He received his scientific training at Bradford Technical College. After some years of analytical experience he became Senior Analyst to Lipton, Ltd., and later, Chemist to C. & T. Harris (Calne), Ltd. In 1937 he became Manager of the Food Cannery of Messrs. J. Travers & Son.

Foster, who died in his 75th year, had been a member of the Society for 46 years. After a period of service as Second Assistant Admiralty Chemist he became Public Analyst for the East Riding of Yorkshire, Grimsby, Beverley and Louth, Official Agricultural Analyst for Grimsby and Gas Examiner for Lincolnshire.

Frankland, who died at the age of 88, was elected an Honorary Member in 1909. He occupied the Chairs of Chemistry at University College, Dundee, and Birmingham, successively. He was elected President of the Institute of Chemistry in 1906 and of the Chemical Society in 1911, and was awarded the Davy Medal of the Royal Society in 1919.

Heywood, who died in his 57th year, had been a member of the Society for 35 years. After studying at University College, London, and long experience in analytical work he was appointed head of the laboratory of Messrs. Courtaulds, Ltd., Castle Works, Flint, and subsequently Technical Manager at the Wolverhampton works of the same firm.

Hinks, who died in his 67th year, had been a member of the Society for 41 years. He received his scientific training at King's College, London, and obtained the B.Sc. degree. He became assistant to Sir Thomas Stevenson, Home Office Analyst and Public Analyst for Surrey, succeeding to the latter appointment on Stevenson's death. He subsequently held a number of other appointments and was also engaged in private practice. For his services as an officer in the Army Ordnance Corps during the 1914–18 war he was awarded the M.B.E. He served on the Council of the Society continuously from 1911 until his death, first as an Ordinary Member, then as Honorary Treasurer, then as President (in 1928–29), and finally as Past President. (Obituary, ANALYST, 1946, **71**, 347.)

Jones, who died in his 84th year, was elected a member of the Society in 1930. Having qualified as a pharmacist, he attended University College, Liverpool, and qualified as A.I.C. in 1894. He became asistant to Professor Campbell Brown and subsequently senior assistant analyst to Professor W. H. Roberts, Public Analyst to the City of Liverpool. He published numerous papers on the microscopy of starches and spices.

Liverseege, who died in his 83rd year, had been a member of the Society for 53 years. He was trained at University College, Nottingham, and Mason College, Birmingham. In

1885 he became assistant to the City Analyst for Birmingham and succeeded to that appointment in 1902, retiring in 1929.

Morrell, who died in his 80th year, became a member of the Society in 1920. From Bradford Grammar School he went to Gonville and Caius College, Cambridge, in 1886. After a period at Wurzburg he returned to his College as a Fellow in 1896 and retained his Fellowship until 1904; then he went to Mander Bros., the well-known paint and varnish manufacturers at Wolverhampton, and retained the position of Chief Chemist until 1930.

Needs, who died in his 63rd year, was elected a member of the Society in 1934. He was trained at the Merchant Venturers Technical College and Bristol University. For some years until 1914 he was assistant to the Public Analyst for Bristol and during the war of 1914–18 served in the R.E. and R.F.A. After that war he became Senior Assistant in the Bristol Corporation Laboratory and in 1930 was appointed Public Analyst for Bristol.

Roberts, who died in his 69th year, had been a member of the Society for 35 years. He graduated at Victoria University and subsequently took the M.Sc. degree. He became City Analyst for Liverpool at the age of 34 and held many other Public Analyst appointments and the Associate Professorship in Public Health Chemistry at Liverpool University. He served the Society as Ordinary Member of the Council, Vice-President in 1934–5 and President in 1938–9. (Obituary, ANALYST, 1947, **72**, 36.)

Watkinson, who died at the age of 60, was elected a member of the Society in 1943. He worked at first in the laboratory of the late Mr. A. H. Allen, and in 1916 went to the Great Central Laboratory, Gorton, Manchester. In 1925 he was moved to Doncaster as a result of the railway amalgamation and was District Chemist there until his retirement.

ORDINARY MEETINGS—Five meetings of the Society were held during the year and the following papers were communicated:

"The Determination of Boron." By E. C. Owen, B.A., M.Sc., Ph.D.

"Separation of the Cobalt Complex of Nitroso-naphthol from other Coloured Metallic Complexes." By E. Boyland, D.Sc.

"Exhibition and Description of Apparatus:

1. For the Control of Delivery from Burettes.
2. A Vacuum-operated Circulating Pump.
3. A Thermostatically-controlled Low-Temperature Bath.
4. For the Continuous Production of Doubly Distilled Water."

By J. T. Stock, M.Sc., F.R.I.C., and M. A. Fill.

"The Microbiological Assay of Amino Acids. I. The Assay of Tryptophan, Leucine, *Iso*leucine, Valine, Cystine, Methionine, Lysine, Phenylalanine, Histidine, Arginine and Threonine." By E. C. Barton-Wright, D.Sc., F.R.I.C.

"The Microbiological Assay of Amino Acids. II. The Distribution of Amino Acids in the Wheat Grain." By E. C. Barton-Wright, D.Sc., F.R.I.C., and T. Moran, D.Sc., Ph.D.

"The Determination of Some Products of Sugar and Molasses Fermentations." By G. G. Freeman and R. I. Morrison.

"The Estimation of the Volatile Matter Content of Propellant Explosives. Part I—The Estimation of Water by an Improved Fischer Method." By T. G. Bonner.

"The Analysis of Barium Carbide." By A. H. Edwards.

"The Fundamental Laws of Polarography." By J. Heyrovsky, D.Sc., Ph.D.

"Reductometric Determination of the Sulphoxide and Amine Oxide Groups." By Mrs. Erica Glynn.

"The Determination of the Composition and Constitution of Ammonium Phosphomolybdate and the Conditions affecting its Precipitation." By W. P. Thistlethwaite, B.Sc., A.R.I.C.

The December Meeting was a Joint Meeting with the Food Group of the Society of Chemical Industry. The subject was "The Application of Statistical Methods to Food Problems," and the following papers were read and discussed:

Introductory Address: "The Inevitability of Statistics." By D. J. Finney, M.A.

"The Use of Statistical Analysis in Research on Food Canning." By W. B. Adam, M.A., F.R.I.C.

"The Evaluation of the Nutritive Value of Animal Feeding Stuffs." By Dr. K. L. Blaxter.

"Application of Statistical Methods in Calculating Proportions of Ingredients in Certain Food Products." By E. H. Steiner, B.Sc., A.R.I.C.

RULES OF SECTIONS AND GROUPS—The Rules of Sections and Groups, prepared by the Special Committee appointed for that purpose, were approved by the Council and issued to all members of the Society with the December number of THE ANALYST.

NORTH OF ENGLAND SECTION—Four meetings have been held during the year. The following papers have been read:

"A Photoelectric Method of Assaying Vitamin A in Margarine." By J. L. Bowen, N. T. Gridgeman, B.Sc., and G. F. Longman, B.Sc., F.R.I.C.

"The Determination of Carotene and Vitamin A in Butter and Margarine." By T. W. Goodwin, M.Sc., A.R.I.C., and Prof. R. A. Morton, Ph.D., D.Sc., F.R.I.C.

"An Application of Photoelectric Spectrophotometry to the Analysis of Mixtures." By Prof. R. A. Morton, Ph.D., D.Sc., F.R.I.C., and Dr. L. A. Stubbs.

"The Determination of the Pyridine Content of Technical Pyridine." By A. Hamer, B.Sc., A.R.I.C., R. Pomfret, A.R.I.C., and W. V. Stubbings, B.Sc., A.R.I.C.

"The Rapid Determination of Sodium in 50 Per Cent. Potassium Hydroxide Liquor, 50 Per Cent. Potassium Carbonate Liquor and Solid Potassium Carbonate." By J. Haslam, M.Sc., F.R.I.C., and J. Beeley.

"The History of Chocolate and Sugar Confectionery." By Dr. L. E. Campbell, F.R.I.C.

"The Determination of Traces of Lead, Zinc and Tin in Phenol." By W. Hutchinson and H. N. Wilson, F.R.I.C.

"Soil Biochemistry." By Dr. H. Lees, B.Sc., A.R.I.C.

There have been good attendances at the meetings. The Hon. Secretary wishes to express his appreciation of the loyal support and assistance accorded to him by the Chairman, Vice-Chairman and members of the Committee during the year.

SCOTTISH SECTION—Three meetings were held during the year, one of them jointly with the Physical Methods Group, at which the following papers were presented and discussed:

"Determination of Vitamins A and A_2 by Photoelectric Spectrophotometry with some remarks on General Principles." By Professor Morton and Dr. A. L. Stubbs.

"Modern Aids to Spectroscopy." By B. S. Cooper, B.Sc., F.Inst.P.

"Application of Spectrographic Analysis to Soil Investigations." By Dr. R. L. Mitchell.

"Spectrographic Analysis of Rare and High Purity Materials." By D. M. Smith, A.R.C.S., B.Sc., F.Inst.P.

"Semimicro Method for the Determination of Magnesium." By James A. Hunter, B.Sc.

"Some Observations on the New Ice Cream Order." By C. W. Herd, Ph.D., B.Sc., F.R.I.C.

The total membership remains the same as for the previous year, *viz.*, sixty-four.

MICROCHEMISTRY GROUP—Two meetings were held during 1946, one in London and one in Cardiff. The latter was held jointly with the Cardiff and District Section of the Royal Institute of Chemistry and the South Wales Section of the Society of Chemical Industry. It was also intended to hold a meeting in Birmingham during September, but owing to circumstances beyond control the arrangements had to be cancelled.

The following papers have been read:

"Chemical Microscopy in Metallurgical Analysis." By Miss I. H. Hadfield.

"A Review of Methods for the Micro Analysis of Gases." By W. A. Kirby.

"Determination of Cyanide by the Picrate Method: A Water-bath for Heating Simultaneously many Tubes of Reactants." By J. G. A. Griffiths and J. K. Whitehead.

"Some Observations on the Kjeldahl Method for the Determination of Nitrogen." By A. E. Beet.

"Methods for the Construction of Microchemical Apparatus." By R. Belcher.

Three meetings will be held in 1947. These include the Annual General Meeting, held in London in January, a meeting at Sheffield in May, and it is hoped a meeting at Cambridge jointly with the Physical Methods Group in September.

The number of Group members is now 181, an increase of 21 since the last Report.

Activities of the Committee—The Committee has met three times during the year. Following the proposal made at the last Annual General Meeting, British Intelligence Objectives Service was asked if a team could be sent to Germany and Austria to report on advances in microchemical methods made during the war. The matter was referred to the Department of Scientific and Industrial Research who agreed to sponsor such a team, and the Committee was invited to make nominations for the team. It was hoped that the trip would take place in September, but it has been postponed several times. The delays are stated to have been due to other bodies demanding representation on the trip, and it is now expected that two teams will travel.

The report on educational institutions teaching microchemistry, prepared by Dr. Cecil L. Wilson, has been published in *Nature* and reprints have been circulated to all members.

A census has also been taken of industrial laboratories using microchemical methods; Dr. Wilson kindly undertook the task at the request of the Committee. A report is now being prepared.

PHYSICAL METHODS GROUP—During the past year the activities of the Physical Methods Group have expanded considerably. The policy announced last year has been continued and each meeting of the Group has taken the form of a symposium on a particular physical method of analysis. Two meetings have been held in London and one each in Edinburgh and Cardiff. The Edinburgh meeting was held jointly with the Scottish Section of the Parent Society and the Cardiff meeting jointly with the Cardiff and District Section of the Royal Institute of Chemistry and the South Wales Section of the Society of Chemical Industry. All the meetings were well attended. The following papers were read at meetings of the Group:

Barker Index Meeting in London on November 28th, 1945.
"The Barker Index." By R. C. Spiller.
"The Utility of the Barker Index in Analytical Chemistry." By M. W. Porter and A. E. J. Vickers.

Chromatographic Analysis Meeting in London on January 26th, 1946.
"General Principles." By F. A. Robinson.
"Partition Chromatography." By R. L. M. Synge.
"Chromatography in the Analysis of Fatty Oils." By K. A. Williams.
"Some Applications of Chromatographic Analysis in Industry." By F. R. Cropper.

Spectroscopic Analysis Meeting in Edinburgh on May 23rd, 1946.
"Photoelectric Spectrophotometry Applied to the Analysis of Mixtures and Vitamin A Oils." By R. A. Morton and A. L. Stubbs.
"Modern Aids to Spectroscopy." By B. S. Cooper.
"Applications of Spectrographic Analysis to Soil Investigations." By R. L. Mitchell.
"Spectrographic Analysis of Rare and High Purity Materials." By D. M. Smith.

Electrometric Analysis Meeting in Cardiff on October 11th, 1946.
"Recent Developments in Apparatus for *p*H Measurements and Electro-titrations." By A. D. E. Lauchlan.
"Some Applications of Electrometric Methods to Analysis." By R. J. Carter.
"Polarisation End Points." By D. P. Evans.

The Publication Committee of the Parent Society has decided to publish in booklet form the proceedings of some of the Group meetings. The booklets covering the Polarography, Chromatography and Spectroscopy meetings will be available shortly.

In May, Dr. F. Wokes resigned from the office of Honorary Secretary and the Committee appointed Dr. J. E. Page to succeed him. The sincere thanks of the Group are due to Dr. Wokes for all the work that he has done since the inception of the Group.

The Committee of the Group has formed a Polarographic Discussion Panel. The objects of the Panel will be to hold and sponsor informal discussions on polarographic analysis. The Committee of the Panel will be elected at the forthcoming Annual General Meeting of the Group.

The Group has been represented on the Vitamin Sub-Committee of the Analytical Methods Committee by Mr. G. F. Lothian and Dr. F. Wokes and on the Barker Index Committee by Dr. J. G. A. Griffiths, Dr. J. H. Hamence and the Honorary Secretary.

The number of the Group members is now 148, an increase of 20 since the last Annual General Meeting.

BIOLOGICAL METHODS GROUP—In the Report of the Provisional Committee which was presented at the first Annual General Meeting of the Group on February 25th last it was stated that the Committee was considering a Draft of Rules for the constitution and conduct of the Groups of the Society which had been put forward for consideration by the Council. A final version of these Rules has now been agreed upon and has been sent to every member of the Group. It is provided in these Rules that the Annual General Meeting of the Biological Methods Group shall be held in December of each year, and it will therefore be convenient for the business year to end on the 30th November. This Report, therefore, covers the period from the first Annual General Meeting up to the 30th November, 1946.

During this period two meetings have been held. The Annual General Meeting was followed by an Ordinary Meeting at which Messrs. N. T. Gridgeman and E. C. Feiller read papers entitled respectively "The Transformation of Metameters, with special reference to Vitamin D Assays," and "Some Remarks on the Statistical Background of Bio-Assays." At a subsequent meeting on the 27th May papers were read by Dr. K. Mather and Dr. G. Pontecorvo on the genetical background of bio-assays, Dr. Mather dealing with the larger animals, and Dr. Pontecorvo with micro-organisms. Both of these meetings were well attended, not only by members, but also by a number of visitors, some of whom have since joined the Society. The membership of the Group is now 87.

PUBLIC ANALYSTS AND OFFICIAL AGRICULTURAL ANALYSTS COMMITTEE—The Committee met during the past year on three occasions. Recommendations have been made in respect of financial matters directly concerning Public Analysts, also in relation to definitions of cocktails and other liquors; some of the latter have since been adopted in part by the Ministry of Food. Two Bulletins have been issued to Public Analysts and a quantity of routine business transacted.

ANALYTICAL METHODS COMMITTEE—Reports of good progress have been received from sub-committees. The appeal published in THE ANALYST (1946, p. 300) to assist the work of the Standard Methods Committee on compilation of a bibliography met with a very satisfactory response and much progress has been made. Considerable work has been done by the Sub-Committees on Vitamin Estimations, Gum Tragacanth and Determination of Metallic Impurities in Foodstuffs.

Two reports from the Committee have been published during the year, *viz.*

"The Determination of Phenols in Soaps." ANALYST, 1946, p. 301.

"Report on the Microbiological Assay of Riboflavine and Nicotinic Acid." ANALYST, 1946, p. 397.

During the year the Committee lost the services by resignation of W. H. Simmons as Hon. Secretary, a position he had held since the inception of the Committee, in 1924, but he remains an active member. Mr. Simmons, whose valuable work for the Committee is greatly appreciated, has been succeeded by Dr. D. C. Garratt.

HON. TREASURER'S REPORT—The financial position for 1946 is satisfactory inasmuch as the income balances the expenditure. But it is the view of the Hon. Treasurer that, having regard to the probability of increased expenditure, due mainly to the increasing activities of the Society, and to the increasing cost of publication of THE ANALYST, it is desirable that the future commitments of the Society should now be critically examined.

THE ANALYST—At the beginning of 1946 the restricted consumption of paper permitted to THE ANALYST by the Paper Control made it difficult to accommodate the increasing number of original papers offered for publication, and some restriction of space given to the other sections of the journal became necessary. Later the paper position was greatly eased by a considerable increase in the permitted consumption, granted on the ground that the journal was "primarily concerned with the first publication of original research." The 1946 volume has 600 pages, compared with 486 in the 1945 volume and 388 in 1944. The number of original papers published in 1946 was 80, compared with 56 in 1945 and 40 in 1944. On the other hand there were only 31 Notes in 1946, compared with 45 in 1945 and 39 in 1944; but these are not a very important factor in the distribution of space in the journal. The circulation of the journal was greater by several hundreds in 1946 than in 1945.

A new development this year is the issue, in the form of stiff-covered reprints, of certain important papers or sets of papers from THE ANALYST. Three are on the point of publication, *viz.*, the Symposia of Polarography, Chromatography and Spectroscopic Analysis, respectively, held at the meetings of the Physical Methods Group.

In consequence of the increasing quantity of matter in THE ANALYST and the need for more efficient organisation of the Abstract section of the journal, it has been found necessary to augment the editorial provisions hitherto made. The Publication Committee have been fortunate in obtaining for this purpose the part-time services of Mr. F.L. Okell, F.R.I.C., as general Assistant Editor, and Mr. L. S. Theobald, M.Sc., A.R.C.S., F.R.I.C., as Associate Editor responsible particularly for the Abstract section of the journal.

EMERGENCY COMMITTEE—This Committee was appointed in September, 1938, with full power to act on behalf of the Council should a state of emergency warrant it. The Committee has met on several occasions since its appointment. The state of emergency having passed, the Committee was not re-appointed for 1946–47.

CHEMICAL COUNCIL—The Society has been co-opted on the Chemical Council and the President and Honorary Treasurer have been appointed as representatives of the Society on the Council.

ROYAL INSTITUTE OF CHEMISTRY—Dr. G. Roche Lynch, Past President of the Society, was elected President of the Royal Institute of Chemistry in March, 1946. The Council has offered its congratulations to Dr. Roche Lynch on this high distinction.

BRITISH STANDARDS INSTITUTION—Dr. W. F. Elvidge was appointed representative of the Society on Committee C/8 and Sub-Committee C/8/1B of the British Standards Institution.

BUREAU OF ABSTRACTS—Mr. A. L. Bacharach was appointed a representative of the Society on a Committee of the Bureau to report on the suggestion to issue separate sets of abstracts covering both the scientific and technical aspects of foods and nutrition.

INSTITUTION OF WATER ENGINEERS—Dr. J. H. Hamence and Mr. S. E. Melling were appointed representatives of the Society on a Joint Committee on the standardisation of methods of chemical analysis of potable waters.

The Office of the Society is available for meetings of small committees; for the accommodation of larger committees the Council again desires to record its thanks to organisations and to members of the Society.

G. W. MONIER-WILLIAMS, *President*
LEWIS EYNON, *Hon. Secretary*

Address of the Retiring President

G. W. MONIER-WILLIAMS, O.B.E., M.C., M.A., PH.D., F.R.I.C.

IT is my privilege on retiring from the chair to address the members of this Society and I propose, with your permission, to follow the usual custom of referring shortly to some of the Society's activities during my term of office and then discussing at greater length a subject of particular interest to many of our members.

The chief events of the past two years are fully recorded in the Council's annual reports, but there are a few matters to which special reference should be made. We have been most unfortunate during this period in losing three of our Past Presidents, Evans, Roberts, and Hinks, to all of whom the Society owes a great debt of gratitude for the work they did for us. Edward Hinks was for many years honorary treasurer before succeeding to the chair, and the value of his work for the Society can hardly be over-estimated. I personally used often to consult him on difficult or controversial matters concerning our profession and could always count on getting thoroughly sound and practical advice. He was ready at all times to take the utmost trouble in the service of the Society, and there have been few whose influence in our Council was greater or whose loss has been more deeply felt. Roberts and Evans also had been active in promoting the interests of the Society and were particularly associated with the work of the North of England Section.

I cannot let this occasion pass without expressing the great pleasure we feel in Dr. Bernard Dyer's continued presence among us. We were able the other day to congratulate him on his 91st birthday, and I can assure him, on behalf of every member of the Society, that we are extremely proud of our distinguished original member and grateful to him for the close and practical interest which he continues to take in the affairs of the Society.

The past year has been remarkable for the success which has attended the proceedings of the three subject groups. The Microchemical Group under the chairmanship of Professor Briscoe, the Physical Methods Group under Mr. Chirnside, and the Biological Methods Group under Mr. Bacharach, have been ably guided through the hazards of infancy and have become firmly established as active and progressive members of the analytical body.

In the coming years the problem of chemical publications is bound to become more and more acute, and the task of our Publication Committee, under Dr. Nicholls, in maintaining the high standard and reputation of THE ANALYST, will become still more exacting. A particularly difficult problem is that of abstracts of papers published in other journals. Our policy is to select our material very carefully over the whole range of analytical chemistry and to make the abstracts full enough to be of real value in the laboratory. This policy makes increasing demands upon the skill and judgment of the editorial staff. We have lately enlarged this staff considerably, and I think we may feel confident that they will prove fully equal to the task before them. In this matter of abstracts we shall be wise to co-operate closely with other publishing bodies, and members of our publication committee have already held preliminary discussions on this subject with representatives of the Bureau of Abstracts. Another important step in the direction of co-operation is the co-option of the Society to membership of the Chemical Council, a position which secures to us a voice in matters of general policy and administration.

The Public Analysts Committee, under the chairmanship of Dr. Cox, recently drafted recommendations on terms and conditions of appointment, for issue by the Council to local authorities. These recommendations have gained sympathetic response and improved conditions in a number of cases, but many authorities still seem unwilling or unable to recognise the heavy responsibility and exacting nature of the work and the long scientific training required. With the emphasis now laid upon sound nutrition as the basis of health, a highly qualified and efficient public analyst service, working under conditions which will attract the ablest men, will become of ever greater importance to the country.

With the end of the war the Analytical Methods Committee has again become active. Some valuable reports have been published, notably one on the microbiological determination of riboflavine and nicotinic acid. A sub-committee is also engaged on a bibliography of published approved and standard methods, which should be extremely useful to analysts in this country. The thanks of the Society are due to Dr. Hughes and his colleagues on the committee, and also to the members of the many sub-committees, for the immense amount of arduous voluntary work which they give so willingly. The reports of this committee contribute very greatly to the high reputation of THE ANALYST both in this country and abroad.

We have been concerned during the past two years with the teaching, or, as some would say, the lack of teaching, of analytical chemistry in university courses. This is a very difficult question and perhaps we, as analysts, run the risk of being accused of undue bias. We may remember, however, that our illustrious former honorary member, Professor Fresenius of Wiesbaden, in the introduction to his well-known book on chemical analysis, emphasises the fact that "chemistry owes to quantitative analysis its elevation to the rank of a science." Further he says that "quantitative analysis forms the strongest and most powerful lever for chemistry as a science, and not less so for chemistry in its applications to the practical purposes of life, to trades, arts and manufactures." I am sure that the truth of these words, written over 70 years ago, is recognised by those responsible for chemical education in this country. Many of us feel, however, that more attention should be given to the systematic teaching of accurate quantitative analysis. Perhaps in these days of overloaded undergraduate courses the answer to the question is to be found in Professor Fresenius' further observation that "the pursuit of this branch of chemistry requires considerable expenditure of time."

I should like to take this opportunity of thanking those who replied so fully to my inquiries upon this subject. Their views have been submitted for consideration to the Chemistry Education Advisory Board, and there are indications that they may meet with a favourable response.

The year 1945 saw the end of the war and it would have been appropriate for me to have attempted a comprehensive survey of the impact of the war years upon this Society and its work, such a survey as was given in a masterly way by Dr. Samuel Rideal at the end of the previous war. Even from the food standpoint alone, however, it would be difficult to do justice to this theme. One would have to deal with food substitutes, food standards, food labelling, the contributions of analytical chemistry to the solution of nutrition problems—all the scientific and administrative activities of the last seven years which have added so greatly to the work and responsibilities of the public analyst. Perhaps it will be more useful if, instead of attempting to cover such a wide field, I make some observations, and I hope some constructive observations, upon one aspect of food administration which has not received much attention during the war.

In all food legislation we can distinguish two distinct and separate objects, firstly the promotion of fair trading without any particular health significance other than that of adequate nutrition, and secondly the protection of the public against possible injury to health from harmful substances. It is this latter object that I want to discuss—the prevention of risk to health from questionable substances which may gain access to food during its preparation, storage or treatment.

Under Section 8 of the Food and Drugs Act of 1938, power was given to the Minister of Health to make regulations prohibiting or restricting the addition of any substance to food and regulating generally its composition. In 1943, under the Defence (Sale of Food) Regulations, similar powers were given to the Minister of Food, but it is not quite clear from the wording of the regulations, or of the explanatory White Paper, which of these Ministers was expected to take action where contamination is concerned. Practically all the orders made by the Minister of Food during the war have been concerned with fair trading or adequate nutrition. There has been some relaxation of the Preservative Regulations of 1925, and quite recently an order concerning edible gelatin, but with these exceptions no orders controlling potentially toxic substances in food have been made either under the 1938 Act or under the Defence (Sale of Food) Regulations. It seems doubtful whether this purely health function will in future be discharged by the Ministry of Health or whether it will be handed over to the Ministry of Food, as appears to have been done with edible gelatin and with fluorine in acid calcium phosphate. Whichever department is ultimately responsible for control of this kind there are, I think, three main principles which should govern any action taken.

If we set out to devise limits of tolerance for toxic substances in food, the chief consideration, obviously, is the effect on health of the small quantities in question, and here we meet with the first and greatest difficulty—what is to be regarded as injurious to health? Owing to the complexity of the processes taking place in the body and the extreme difficulty of relating cause and effect, there is usually a very wide range between that which can be accepted without question as being harmless and that which can be shown conclusively to be injurious. A limit might be fixed anywhere within this range and the claim made that the consumer had been adequately protected.

There are many people concerned with the chemistry of food and nutrition who deprecate the placing of any restraint upon trade in these matters unless it can be clearly shown to be necessary in order to prevent injury to health and who hold strictly to the experimental method. If any limit is set for a toxic substance they would put it high in this doubtful range on the ground that there is no experimental evidence that the substance in question is injurious in that amount. At the other extreme there are many who think that any suspicion that a substance may be injurious, even to a minority of the less resistant persons, should be enough to warrant its exclusion altogether from food. They would condemn any treatment of food which may involve the slightest contamination with questionable substances.

As an example of the difficulty of deciding what is or is not injurious we may take lead. We have first to consider what is the safe daily intake of lead on the assumption that it is all absorbed, for this will affect the safety limits for different foods. In spite of the long experience gained with water supplies this point is not yet definitely settled. Some people are more susceptible than others to lead poisoning, women for instance much more so than men. The effects of lead are cumulative: even when ingested in minute amounts it can be stored in the body and again become active under different conditions of health and of dietary habits. Its ultimate effects may not be evident for several years. Then again there is the difficulty of recognising with certainty the milder forms of lead poisoning. Some authorities hold that it is responsible for many anomalous nervous conditions and slight ill health. On the other hand it seems that many people who come into contact with lead industrially may excrete it in relatively large amounts and yet show none of the recognised symptoms of lead poisoning.

When we have arrived at some conclusion as to the limit which should be set for the amount of lead absorbed into the system over any given period we have still to consider what proportion of the lead in food is actually so absorbed. Water, beer and cider seem to give it up much more readily than solid foods. In particular a high intake of calcium in the diet hinders absorption; from foods rich in milk or dried milk, lead, if present, is absorbed only slightly. Phytic acid, also, and pectin may combine with lead and remove it from solution, and there are probably other constituents of food which have a similar effect. Absorption of lead may thus depend to some extent upon the nature of the food, and even upon the sequence of different foods, from day to day.

These are problems for the physiologist, the nutrition specialist and the chemist together, but even the most expert team would be hard put to it to prepare a schedule of limits for lead in different foods which would be free from criticism. A similar uncertainty applies more or less to other potentially toxic substances in food; for instance, antimony or zinc, certain azo dyes as colouring matters, thio-urea as an antioxidant, or methyl bromide as a fumigating agent. Methyl bromide in small doses over a long period has been found to hinder the growth of animals. Can we disentangle from the multitude of factors affecting growth in human beings a possible slight effect due to the use of methyl bromide for fumigating food and its retention by fat? How are we to say at what point a contamination or treatment of food loses its harmless character and becomes suspect?

The Minister of Health has often been asked to give a ruling on specific cases but has rarely, if ever, done so. There is a clear distinction between giving an interpretation of the meaning of the word "injurious" in Section 1 of the Act, which is the prerogative of the Courts, and embodying that interpretation in a regulation under Section 8, which is the privilege of the Minister. Section 7 of the Act lays it down that any addition to food which has been forbidden by regulations is *ipso facto* to be regarded as injurious to health, which seems to solve the problem quite neatly, but unfortunately regulations are always several laps behind public health requirements. The object of Section 7 is, presumably, to uphold the regulations in a possible conflict of expert evidence. I am not aware of any similar provision in respect of orders made under the Defence (Sale of Food) Regulations.

This Society, in a memorandum submitted to the Departmental Committee on the Composition and Description of Food, urged the setting up of a permanent statutory advisory committee consisting of representatives of the Ministry of Health, public analysts, manufacturers and consumers which, among other duties, "should on request state definitely with regard to any ingredient of a food, the interpretation of the term 'injurious to health'." This suggestion, possibly for the reasons I have given, was not favoured by the Departmental Committee, which made an alternative recommendation that any controversial question on which an order is applied for should be referred to an *ad hoc* committee of three independent persons for public inquiry and report. Neither of these suggestions has been adopted. There seems to be a tendency now to refer these questions to the Medical Research Council, but I doubt whether this is the proper body to deal with them.

In difficult and contentious matters of this kind the first instinct of the administrator is to compromise, but we may question whether this instinct is sound where food is concerned. One need not elaborate the fact that food is in a category altogether different from that of any other commodity. We are bound to take into consideration the chances of remote action beyond the reach of experimental proof and to insist upon a wide margin in favour of the consumer. The proper course is to ensure that, within the range in which doubt exists as to the toxicity of any substance in food, the limit of tolerance shall be placed at the lowest possible point, and this, I suggest, should be regarded as a first principle. It is precisely that lack of knowledge, that difficulty of experimental proof, which should make us chary of compromising in this matter.

If this principle is accepted, and if we have succeeded in defining the doubtful range in respect of a particular substance, we have to decide what are the very lowest limits within that range which can reasonably be insisted upon as a practical measure. It must be remembered that food manufacture, packing and distribution are by no means confined to large concerns with ample resources for regular scientific control. Many of the smaller concerns find it hard to keep a constant check on raw materials and to maintain a consistently high standard. In deciding upon appropriate limits we must consider carefully what can be reasonably demanded in all circumstances. I do not suggest for a moment that there should be any allowance for inefficiency; rather that every endeavour should be made to discover what can and what can not be done by different types of producers working under different conditions. It may be that a requirement which seems at the time impossible of fulfilment will act as a stimulus to improvement and will soon become normal practice. It is of course very difficult to arrive at a sound conclusion. The necessity for careful and detailed inquiry has been recognised by successive departmental committees. In the old days of the Foods Department of the Local Government Board, and later of the Ministry of Health, someone with the requisite experience was detailed to investigate and report on a particular contamination or treatment of food as fully as possible, by visits to factories and by making contact with anyone who could give trustworthy information on the subject. Probably this method, in the hands of a competent investigator, gets at the facts more surely than the alternative one of taking evidence from, and cross-examining, interested parties at a committee table. The Interdepartmental Committee on Preservatives in 1923–24 employed both methods, and the subsequent regulations were the result of as full an inquiry as was possible with the limited staff available Even so, several points were missed but, considering the wide field covered by the inquiry, this was perhaps inevitable. We may remember that the Royal Commission on Arsenical Poisoning, whose report stands out as a model of thorough and exhaustive investigation, failed to appreciate the partiality of shell-fish for arsenic.

My second principle then, although it sounds self-evident, is that, when it has been decided on health grounds that control is necessary, the fullest possible investigation should first be made of all the commercial factors involved. This implies of course that an adequate staff should be made available for the purpose, but nowadays one hesitates to suggest any further addition to the public service.

When a decision has been reached on the practical limits to be adopted for any foreign substance in a particular food or foods there remains the question whether these limits or prohibitions should be embodied in regulations, or whether there is any other way of securing their acceptance by the trade and by those concerned with administration. Statutory standards have an immense advantage from the viewpoint of administrative convenience and greatly simplify the whole procedure of Food and Drugs Act work. They are also a safeguard for the manufacturer and distributor, who thus know exactly where they stand and are not so liable to become involved in unforeseen litigation. On the other hand it is very difficult, when framing regulations, to take every factor into account, and clearly impossible to make adequate allowance for changing circumstances. Legal standards have the disadvantage that they are not readily altered and may remain in force long after scientific research and industrial progress have made them virtually obsolete. Nominally it should be an easy matter to amend a regulation—and this is one of the advantages claimed for departmental regulations as compared with Acts of Parliament—but in practice it has proved difficult, even when the weight of evidence has been overwhelmingly in favour of amendment. I am not speaking, of course, of war-time orders which have to deal quickly with constantly changing conditions of supply.

The third principle, therefore, is that any regulations on food matters must allow of ready revision, not only in theory but in practice, as unforeseen contingencies arise.

A common charge against standards is that they tend to become the normal and that while they may induce a general levelling up of poor quality articles there is also a tendency to a levelling down of the better qualities, to the disadvantage of the consumer. If, however, we are able to place the limit for a toxic substance low enough, this objection loses much of its force. Another objection, of course, is the instinctive dislike of official interference in our daily lives. The experience of the last six years has not made us anxious for further restrictions. It will be agreed, I think, that no standard of the type we are discussing should be imposed unless a reasonable case has been made out for it on health grounds. There is always a danger that a demand for standards may be prompted more by a desire for administrative convenience than by health considerations.

There has been much criticism of government by shoals of departmental regulations having the force of law, and it is worth while considering whether in certain cases official pronouncements or recommendations might carry enough weight to make such regulations unnecessary. Arsenic provides a case in point. The limits proposed for arsenic in food by the Royal Commission over forty years ago have never yet been given statutory force except for edible gelatin, and yet they have been accepted without serious question for administrative purposes. In fact the absence of a rigid standard has on occasions been undeniably convenient, as for instance when arsenic of natural origin was discovered in fish and more recently in parsley.

Would similar pronouncements in respect of other potentially injurious substances in food, backed by a sufficient weight of authority, be accepted in the same way as the arsenic limits? I very much doubt it. The recommendations of the Royal Commission followed a serious and widespread outbreak of poisoning in Lancashire, and the general acceptance of the limits then proposed was due more to the shock of that occurrence and the fear of further trouble than to the authority of Lord Kelvin and his colleagues on the Commission. The questions of lead, copper, and antimony, of preservatives and colours, of flavouring vehicles, of fumigating agents, and of antioxidants are more controversial and less clear cut than that of arsenic, and the possible effects on health are not nearly so spectacular. The report of the Departmental Committee on Preservatives, a body just as competent as the Royal Commission to decide the health question, would have had little influence on the use of preservatives if its findings had not been crystallised into regulations. The report by itself would undoubtedly have been of assistance to those whose task it is to interpret the meaning of the word "injurious" in the Act, and indeed several of the Local Government Board and Ministry of Health reports have been of great value in this way; but the regulations and standards which followed the findings of the Preservatives Committee have had a definite and salutory effect on the quality of food in this country and on the conditions under which it is prepared and stored. Moreover, it is doubtful whether any advisory body would give sufficiently close attention to these problems, especially the more controversial of them, if their advice was to be treated only as an opinion to be contested and possibly over-ruled. Such advice would have to be definitely associated with a proposal for an order or regulation.

In the absence of statutory standards certain administrative methods have been adopted with more or less success to secure some sort of control. Thus medical officers at the ports, using their powers under the Imported Food Regulations, agreed a few years ago on limits for toxic metals in some imported foods. The importer could, of course, appeal to the Courts if he felt aggrieved, but in practice he seldom did so. This Society has, in a few instances, formulated its own standards, and local authorities often bring pressure to bear upon manufacturers and distributors to accept certain criteria of purity in respect of particular foods.

These isolated efforts only touch the fringe of the problem. During the recent period of stringency and short supply many things have of necessity been tolerated which will demand careful reconsideration on return to more normal conditions. It is clear that if any contamination or treatment of food is suspected of being in any way injurious, the consumer's interest demands that it should be effectively regulated. Public analysts have always been insistent upon this. At present they are in the invidious position of having, in the first instance, to apply their own standards in order to carry out their duties under the Act. They are justified in asking that they should be relieved of this responsibility and that it should be undertaken by some department of State through an advisory body combining the necessary scientific, commercial and administrative experience and authority.

In conclusion, I should like to express my thanks to the officers, council, and members of this Society for the kindness and consideration which they have at all times shown to me, and to accord to you, Mr. President, my best wishes for your term of office.

The Oxidation of Nitrogen during the Micro-Combustion of Organic Substances

By A. E. HERON

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According to Pregl,[1] amino, imino, and similar compounds yield on combustion, in addition to carbon dioxide and water, elementary nitrogen, or at the most, nitrous oxide. In their paper on "A New Technique for the Ultimate Micro Analysis of Organic Compounds," Belcher and Spooner[2] state that it depends on how the nitrogen is combined whether or not oxides of nitrogen are formed, but recommend the use of a bubbler containing acid dichromate or permanganate, with all compounds containing nitrogen, until information is available as to which compounds do and which do not form oxides of nitrogen.

In March, 1944, we were comparing the standard macro- and the Pregl micro-method for the analysis of coal, and found that the results obtained with 5- to 7-mg. samples and

Pregl's method were not greatly divergent from those obtained with 150-mg. samples by the standard macro-method. The results obtained with a modified form of the combustion technique described in the paper referred to,[2] but without the chromic and sulphuric acid bubbler, were all somewhat higher than those obtained by our standard macro-method. These results were published in *J. Inst. Fuel*,[3] and the suggestion was made that the discrepancy might be due to retention, by the reagent in the carbon dioxide absorption tube, of oxides of nitrogen produced from the nitrogen contained in the coal. It was subsequently shown that oxides of nitrogen could be readily detected in the products of combustion of a 5-mg. sample. Analyses of the same sample of coal when a bubbler containing chromic and sulphuric acids was used gave results that agreed tolerably well with those obtained by the Pregl micro- and our standard macro-method. In all the experiments on the micro scale the sample was burned in a platinum boat. In the method of Belcher and Spooner[4] for the rapid simultaneous determination of carbon, hydrogen, sulphur, and chlorine in coal, on the *macro* scale, samples are burned in a rapid stream of oxygen at a temperature of 1300° C. No special provision is made for the removal of oxides of nitrogen, for, according to the authors, under the conditions employed, no oxides of nitrogen are formed, the nitrogen being evolved completely as elementary nitrogen gas.[3] Thus, although the influence of nitrogen on the macro scale is apparently insignificant, our results suggested that the effect of nitrogen on the micro scale was important and required further investigation.

In a private communication, Belcher stated that he had carried out some experiments on the macro scale, using porcelain and platinum boats to contain the sample of coal, and had found that if platinum was used oxides of nitrogen could be detected in the products of combustion, but that he could find none when porcelain was used. He also said that tests on the micro scale, with use of a train packed with silica chips, such as we were using in our modification of the unpacked tube technique, indicated that nitrogen oxide formation could be detected to a small extent when coal was burnt in a porcelain boat, and more readily when a platinum boat was used.

It was thought that the problem should be investigated quantitatively and that the results obtained by examining the products of combustion of various nitrogen-containing organic substances would be of real value. According to Kirner,[5] "oxidation of the nitrogen-containing thermal decomposition products by the excess of oxygen present in the combustion tube at temperatures from 600° C. down to 180° C. would yield only nitrogen peroxide and nitrogen. The proportion of these two substances which finally results under a given set of conditions should primarily be a function of the manner in which the nitrogen is linked in the molecule." The purpose of our investigation was therefore to find out how much oxidation of nitrogen occurs during the combustion of compounds containing nitrogen. The determination of the extent to which this occurs resolves itself into the finding of a suitable absorbent, the oxidation of the absorbed gas, and the evaluation, by a suitable method, of the nitrate produced.

EXPERIMENTAL

The silica combustion tube was of the usual type with side arm; it was heated by an electric furnace giving a temperature of 1000° C. at the centre. Toward the exit end of the tube was a 2½-inch roll of silver gauze, so placed that about half of it was heated directly by the furnace winding. The exit end of the tube was heated by a copper block, maintained at 130° to 160° C. to prevent condensation of water. In some of the experiments the portion of the tube that was heated in the furnace was packed with silica chips, but in others the tube was free from packing. A pre-heater packed with silica chips was used. For the determination of the "oxides of nitrogen" it was decided to use a colorimetric method which depended upon the nitration of an organic compound, rather than upon an oxidation effect as does the diphenylamine test. Soda asbestos as used for the carbon determination was tried as absorbent for the nitrogen peroxide, but replicate tests for nitrate on weighed amounts gave widely different results. It was found that when the gases were passed through a spiral bubbler containing sodium hydroxide solution, absorption of carbon dioxide was not complete; it could be detected in the gases after they had passed through the spiral bubbler. A vacuum technique was also tried by means of which all the gases obtained during the combustion could be submitted to the action of an absorbent solution. Heavy walled bottles of about 750 ml. capacity were obtained and each fitted with a rubber bung carrying a single-way tap, the end of which was gradually tapered to the same diameter as the exit of the combustion tube. When a determination was to be made, a small volume of suitable absorbent was measured into a bottle, which was then evacuated with a good water-pump, and connected to the train. The rate of flow of oxygen was controlled by adjustment of the tap fitted to the bottle. In the earlier experiments a bubble-counter was used to indicate the rate of flow of oxygen, but later a White - Wright flow meter[6] was substituted.

Alkaline permanganate was first tried as absorbent. This is a recognised absorbent for nitric oxide and nitrogen peroxide. After the gases had been in contact with this solution for 3 hours, it was acidified with 20 per cent. sulphuric acid and warmed to about 40° C. for 15 minutes to oxidise nitrite. Hydrogen peroxide was then added to destroy the excess of permanganate and followed by a slight excess of sodium hydroxide. The sample was now boiled and filtered, and the filtrate evaporated to dryness. Two ml. of a 1·25 per cent. solution of sulphosalicylic acid in 50 per cent. sulphuric acid were added and the solution was heated on a water-bath for 30 minutes. It was then diluted, 25 ml. of 10 per cent. sodium hydroxide solution were added, the volume was made up to 50 ml. and the optical density was measured on the Spekker absorptiometer at 4360A. In a number of tests trouble was experienced owing to precipitation of manganese after the final addition of sodium hydroxide, and the blank values obtained were not consistent. A similar lack of agreement on control tests was observed when the absorbent used was 80 per cent. sulphuric acid containing sulphosalicylic acid. It is possible that in this case there was some carbonisation of the reagent.

The use of 2:4-xylenol was then considered. When this reagent is used, the nitro compound may be steam distilled from the solution in which it is formed, and thus separated from substances that might interfere with the measurement of its optical density. At the same time, in order to reduce the number of reagents employed to a minimum and to keep the technique as simple as possible, it was decided to use as absorbent ceric sulphate in sulphuric acid, a reagent which rapidly oxidises nitrite to nitrate at room temperature. With these modifications to the method, there was no difficulty in obtaining agreement in blank tests.

The procedure was as follows—

The electric furnaces were switched on and the temperature of the copper block adjusted. The oxygen flow was adjusted to a rate of about 35 ml. per minute. Three ml. of 0·1 *N* ceric sulphate solution in 12 per cent. sulphuric acid were measured into the vacuum bottle, which was evacuated by means of a good water pump, and connected to the train. The weighed sample was then introduced into the combustion tube and this was securely stoppered with the rubber bung. The tap of the vacuum bottle was cautiously opened until a rate of flow of oxygen of 25 ml. per minute was indicated by the bubbler or flow meter. The sample was then burned off and the products of combustion swept forward in the usual way, the oxygen rate being maintained at 25 to 30 ml. per minute throughout, by adjustment of the tap. At the end of twenty minutes the tap was closed and the bottle disconnected from the train. The inlet tube was washed with 2 ml. of 20 per cent. sulphuric acid without removal of the stopper from the bottle, the contents of which were still under slightly reduced pressure. The bottle was then rotated frequently over a period of 1 hour, and at the end of this time the stopper was removed. 0·2 *N* Ferrous sulphate was added till the colour of the ceric sulphate was just discharged, and then followed a dropwise addition of 0·1 *N* potassium permanganate until a barely perceptible pink colour was obtained. The permanganate, of which only 1 or 2 drops are required, was used as an indicator, ceric sulphate itself not being sufficiently intensely coloured. Five ml. of a 1 per cent. solution of 2:4-xylenol in dilute sodium hydroxide solution were added, followed by 35 ml. of sulphuric acid of sp.gr. 1·76. The temperature while this was being added was not allowed to exceed 35° C.; the solution was at this temperature for 30 minutes. At the end of this time the contents of the bottle were washed into a steam distillation apparatus, with approximately 150 ml. of water. The nitro compound and excess of 2:4-xylenol were distilled into 1 ml. of *N* sodium hydroxide. The distillate was made up to a given volume and its optical density determined on a Spekker absorptiometer. The mercury lamp was used with Chance No. 6 and Wratten No. 50 filters (4360A).

RECOVERY OF NITROGEN—

Dinitrobenzene was burned, the products of combustion were collected and the oxides of nitrogen formed determined as described above. Five determinations were made, two with a boat of fireclay, and three with a boat of platinum. The tube contained only the silver coil. The results are given in Table I. This and the subsequent tables show the conditions of each experiment, the weight of sample taken and the quantity of "oxides of nitrogen," calculated as NO_2, which was collected in the ceric sulphate solution. This is also shown as a percentage of nitrogen on the sample. Finally the proportion of nitrogen collected as NO_2 is shown as a percentage of the total nitrogen present.

TABLE I

COMBUSTION OF DINITROBENZENE, 16·7 PER CENT. N

Expt. No.	Conditions of test	Boat	Wt. of sample mg.	Nitrogen absorbed: Calc. as mg. NO_2	Calc. as N % of sample	Calc. as N % of total N
1	Combustion tube contained only silver coil. Oxygen rate 25 to 30 ml. per min.	Fireclay	6·376	3·29	15·7	94·2
2		,,	7·045	3·75	16·2	97·2
3		Platinum	7·310	3·88	16·1	96·6
4		,,	8·678	4·54	15·8	94·8
5		,,	8·960	4·05	13·7	82·2

These results were considered satisfactory for the purpose of this investigation and the method was used for all the subsequent work.

THE COMBUSTION OF COAL—

A sample of Craghead coking coal which contained 1·26 per cent. of nitrogen, as determined by the Kjeldahl method, was used for the work on coal. Weighed amounts of this sample were burned under different conditions, and the results are recorded in Table II. Oxides of nitrogen were produced in all experiments. The results when the tube was packed with silica chips and contained the silver coil ranged from 9·5 to 30·2 per cent., when the boat was of fireclay. When the platinum boat was used, the results ranged from 10·3 to 31·4 per cent. In each case the distribution of results was fairly even over the whole range. When the tube contained only the silver spiral, the results with the porcelain boat ranged from 7·9 to 17·5 per cent. and with the platinum boat from 11·8 to 24·6 per cent. It is of interest that W. R. Kirner,[7] in a series of experiments for the direct simultaneous micro-determination of carbon, hydrogen, and oxygen in coal, found that correct oxygen values were obtained when it was postulated that 59 per cent. of the nitrogen present was converted into nitrogen peroxide and 41 per cent. to elemental nitrogen. This figure for nitrogen peroxide is much higher than any of the results we have obtained.

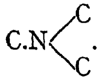
TABLE II

COMBUSTION OF CRAGHEAD COKING COAL, 1·26 PER CENT. N

Expt. No.	Conditions of test	Boat	Weight of sample mg.	Nitrogen absorbed: Calc. as mg. NO_2	Calc. as N % of sample	Calc. as N % of total N
6	Tube packed with silica chips and containing silver coil. Oxygen rate 25 to 30 ml./min.	Fireclay	10·843	0·105	0·30	23·8
7		,,	9·822	0·039	0·12	9·5
8		,,	9·864	0·063	0·19	15·1
9		,,	10·022	0·066	0·20	15·9
10		,,	10·412	0·049	0·15	11·9
11		,,	10·466	0·095	0·28	22·3
12		,,	9·749	0·079	0·25	19·9
13		,,	10·822	0·102	0·28	22·3
14		,,	12·240	0·151	0·38	30·2
15		Platinum	9·640	0·098	0·31	25·0
16		,,	10·050	0·066	0·20	15·9
17		,,	8·654	0·049	0·18	13·5
18		,,	10·068	0·043	0·13	10·3
19		,,	10·083	0·085	0·26	20·6
20		,,	9·843	0·138	0·38	30·2
21		,,	10·039	0·121	0·37	29·4
22		,,	9·840	0·128	0·40	31·4
23		,,	10·422	0·135	0·39	30·9
24	No silica chips, otherwise as above.	Fireclay	9·642	0·068	0·22	17·5
25		,,	10·625	0·061	0·19	14·3
26		,,	10·053	0·032	0·10	7·9
27		Platinum	10·383	0·059	0·17	13·5
28		,,	10·545	0·106	0·31	24·6
29		,,	9·842	0·049	0·15	11·8

COMBUSTION OF PURE ORGANIC COMPOUNDS—

Table III shows the results of combustions carried out on various pure organic compounds: (*a*) carbazole, (*b*) pyridine, (*c*) xylidine, (*d*) dimethylaminoazobenzene (dimethyl yellow) and (*e*) urea. With all of them oxides of nitrogen were formed in variable amounts. Carbazole, for example, yielded from 7·4 to 32·2 of its total nitrogen as oxides and there was no marked difference in the order of magnitude of the results according as fireclay or platinum boats were used. With pyridine, again, the results show no marked differences attributable to any particular condition in the tests. Dimethyl yellow contains two types of nitrogen linkage, *viz.*, C.N:N.C and



$C.N\begin{matrix} C \\ C \end{matrix}$. The amounts of nitrogen oxides formed from urea were consistently small. One result amounting to 48 per cent. of the total nitrogen was obtained, but as this figure was not approached in any subsequent test it has been rejected

TABLE III

COMBUSTION OF PURE ORGANIC COMPOUNDS

Expt. No.	Conditions of test	Boat	Weight of sample mg.	Nitrogen absorbed: Calc. as mg. NO_2	Calc. as N % of sample	Calc. as N % of total N
	(*a*) COMBUSTION OF CARBAZOLE, $C_6H_4.NH.C_6H_4$, 8·4% N					
30	Tube packed with silica chips and containing silver coil and boat with sample.	Fireclay	10·328	0·490	1·43	17·0
31		,,	9·851	0·259	0·80	9·5
32		,,	8·502	0·756	2·70	32·2
33		,,	8·306	0·665	2·44	29·2
34		Platinum	8·511	0·598	2·14	25·4
35		,,	10·522	0·216	0·62	7·4
36	Tube contained only silver spiral and boat with sample.	Fireclay	10·310	0·484	1·40	16·7
37		,,	12·780	0·319	0·76	9·1
38		Platinum	10·550	0·484	1·39	16·5
39		,,	11·890	0·298	0·74	8·8
40	Tube contained only boat with sample.	Fireclay	10·497	0·362	1·04	12·4
41		Platinum	10·287	0·328	0·98	11·7
	(*b*) COMBUSTION OF PYRIDINE, C_5H_5N, 17·7% N					
42	Tube packed with silica chips and containing silver coil and boat with sample.	Fireclay	10·684	1·260	3·60	20·3
43		Platinum	6·990	0·415	1·80	10·3
44		,,	8·690	1·015	3·58	20·2
45	Tube contained silver coil and boat with sample.	Fireclay	10·420	0·756	2·20	12·5
46		,,	8·116	1·882	7·08	40·0
47		Platinum	5·424	0·684	3·84	20·5
	(*c*) COMBUSTION OF XYLIDINE $(CH_3)_2C_6H_3NH_2$, 11·5% N					
48	Tube packed with silica chips and containing silver coil and boat with sample.	Platinum	11·100	0·342	0·94	8·1
49		,,	8·624	0·495	1·75	15·2
50		Fireclay	9·961	0·645	1·96	17·0
51		,,	10·727	0·529	1·50	13·1
	(*d*) COMBUSTION OF DIMETHYL YELLOW, $C_6H_5N:N.C_6H_4N(CH_3)_2$, 18·65% N					
52	Tube containing silver coil and boat with sample.	Fireclay	7·504	0·379	1·54	8·3
53		,,	6·760	0·551	1·98	10·6
54		Platinum	8·520	0·437	1·98	10·6
	(*e*) COMBUSTION OF UREA, $CO(NH_2)_2$, 46·6% N					
55	Tube packed with silica chips and containing silver coil and boat with sample.	Fireclay	9·186	0·349	1·31	2·8
56		,,	11·045	0·402	1·07	2·3
57		Platinum	9·900	0·414	1·27	2·8
58		,,	8·765	0·609	2·11	4·6
59	Tube contained silver coil and boat with sample.	Fireclay	10·740	0·168	0·48	1·0
60		,,	12·928	0·312	0·73	1·6
61		Platinum	11·701	0·282	0·73	1·6
62		,,	9·854	0·175	0·54	1·2

Finally, several experiments were carried out in which air was passed through the train with a platinum or fireclay boat in position. With a platinum boat the results obtained when 500 ml. of air were passed along the tube at a rate of 25 to 30 ml. per minute showed that oxides of nitrogen equivalent to 0·01 to 0·02 mg. of NO_2 were produced and that when fireclay boats were used the results varied between 0·003 and 0·01 mgm. It should be added here, that the control tests on reagents and blank tests for the combustions in oxygen were deducted in every case, in the calculation of results.

DISCUSSION—

The investigation has shown that when compounds of different types containing carbon, hydrogen and nitrogen are burned in a stream of oxygen, appreciable amounts of oxides of nitrogen are produced. The results obtained from the combustion of coal indicate that although the material used for the boat may have some effect, it is of minor importance, and the effect of the surface provided by the silica chips is somewhat greater.

In the written discussion on the papers by Belcher and Fenton on "Methods for the Determination of Carbon and Hydrogen in Coal and their Practical Utility"[3] results obtained on the same sample of coal by the B.S. method[8] and the open tube technique[4] are quoted by Edwards and Vahrman. These results are in substantial agreement, but it should be noted that in neither of these methods is special provision made for the removal of oxides of nitrogen from the products of combustion, and thus it is unsound to deduce from these results that oxidation does not occur. Oxidation to the extent indicated in Table II would result in the carbon values being high by approximately 0·9 times the amount of nitrogen absorbed (*i.e.*, from 0·11 to 0·36 per cent.). An error of this magnitude would be of no special significance in the analysis of coal.

The subordinate effect of the silica packing can also be traced in the results obtained by the combustion of the pure organic compounds, but with these the effect of replacing the fireclay boat by one of platinum was not marked. The oxidation of the nitrogen probably takes place at the instant of ignition. The rapidity with which a given weight of sample is consumed is not easy to control as it is influenced by several factors, *e.g.*, the thickness and length of the layer in the boat and the temperature of the tube at the instant of ignition. These factors may be responsible for the variation in the results obtained on each compound. Up to 40 per cent. of the total nitrogen may be oxidised during the combustion.

Kirner,[5] who used a combustion tube packed with copper oxide, states that in cases where nitrogen is linked to hydrogen and carbon, the conversion to nitrogen peroxide is 26 per cent. and for nitriles, heterocyclic nitrogen compounds and nitro compounds, the conversion is 59 per cent. Our results indicate that for the amino compounds examined oxidation may vary between 1 and 17 per cent. of the total nitrogen, and for heterocyclic compounds the range lies between 7 and 40 per cent. This represents less conversion of nitrogen to nitrogen dioxide than Kirner's work suggests should take place when this type of compound is burned in oxygen. For dinitrobenzene the amount of nitrogen dioxide absorbed represented between 82 and 97 per cent. of the nitrogen present, a much higher value than that quoted by Kirner.

Our experiments amply confirm Pregl's statement[1] that "metallic silver is quite unsuitable for the reliable absorption of the nitrogen oxides," but we have not confirmed his statement that "amino and similar compounds yield on combustion elementary nitrogen only, or at most possibly nitrous oxides."

It is evident that if carbon and hydrogen are to be determined on organic substances containing amino, heterocyclic, diazo or nitro nitrogen provision must be made to remove oxides of nitrogen from the products of combustion, when combustions are carried out on the micro scale.

SUMMARY—

Experiments on the micro scale have been performed to determine the extent to which oxides of nitrogen are produced when organic compounds containing carbon, hydrogen, and nitrogen are burned in oxygen. Almost the whole of the nitrogen present in nitro compounds is recovered as oxides of nitrogen; when nitrogen is not linked to oxygen, between one and forty per cent. of the total is oxidised. Thus, when carbon and hydrogen are to be determined on compounds containing nitrogen on the micro scale, provision must be made to remove oxides of nitrogen completely from the products of combustion.

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IMPERIAL CHEMICAL INDUSTRIES, LIMITED
BILLINGHAM DIVISION
RESEARCH DEPARTMENT, ANALYTICAL LABORATORY
BILLINGHAM, CO. DURHAM

October, 1946

DISCUSSION

Mr. R. BELCHER said that Mr. Heron's investigation constituted a valuable contribution to our knowledge of the behaviour of nitrogen-containing compounds when burnt in oxygen. He felt, however, that much still remained to be explained. The combustion technique used by Mr. Heron was one of a series of methods that have been developed, using what was called the "empty-tube" technique. The first of these methods to be developed was that for the simultaneous determination of sulphur and chlorine in coals (A. E. Beet and R. Belcher, *Fuel*, 1940, **19**, 42). This was a macro method using 0·5 to 1·0 g. of sample. In the initial stages of the work it was assumed that nitrogen oxides would be formed from oxidation of the nitrogen in the coal, and a titrimetric method was sought that would be specific for the sulphate ion, rather than an alkalimetric method, because it was believed that nitric acid would be formed. Since none of the titrimetric methods for sulphate examined was reliable it was decided to test whether or not nitrogen oxides were formed when the coal was burnt under these conditions, that is, in an empty porcelain tube at a temperature of 1350° to 1400° C. No trace of nitrogen oxides was detected by qualitative tests with the brucine reagent. In the method finally adopted sulphur oxides and chlorine were absorbed in hydrogen peroxide to give sulphuric and hydrochloric acids respectively. After determination of the total acidity, chloride was determined titrimetrically and the sulphur figure found by the difference between the two readings. Since the results gave good agreement with the B.S. methods, further evidence—although indirect—was thus supplied that nitric acid was absent, for otherwise the results would have been high. That there was no loss of chlorine or sulphur oxides, compensated by nitric acid absorption was shown by carrying out gravimetric determinations for sulphate and chloride. This method had been adopted by many laboratories in the coking industry and had been recommended as a standard method for routine testing within the industry. Attention was next turned to the determination of carbon and hydrogen in coals and a macro-method in which carbon, hydrogen, sulphur, and chlorine were determined simultaneously was evolved (R. Belcher and C. E. Spooner, *Fuel*, 1941, **20**, 130). If nitrogen oxides were formed, one would have expected the carbon figures to have been unduly high, whereas all results were in accord with those obtained by the B.S. method. Again, this method or a modification of it was used in other laboratories, which, he believed, had satisfied themselves, both by qualitative tests and indirectly by comparing the results with those given by the B.S. method, that nitrogen oxides are not formed. This method had given so much satisfaction and possessed so many advantages over the B.S. method that the British Standards Institution was to examine it with a view to its recommendation in the standard specifications. So far the methods discussed were carried out on the macro-scale and only applied to coals. Later the same technique was used for the analysis of organic compounds on both the macro- and the micro-scale at a temperature of 800° C. during the combustion. The speaker and his collaborators concerned themselves only with the application of the method to organic compounds and did not apply it to coals. Had they done so they would certainly have assumed that nitrogen oxide formation was non-existent, for it was reasonable to assume that a mere reduction in the scale of working would not cause nitrogen oxide to be formed. The few nitrogen-containing compounds that were examined appeared to bear out Pregl's statement, as quoted by Mr. Heron. When the nitrogen was already linked to oxygen, nitrogen oxides were formed. With other combinations such as an amino group, there was no evidence of nitrogen oxide formation. However, it was felt that a more thorough investigation of this aspect was required, hence the cautious statement with which Mr. Heron prefaces his account, that a bubbler containing an absorbent for nitrogen oxides should always be included if the compound contains nitrogen. Mr. Heron's investigation appeared to indicate that this precaution must now always be taken. It was the behaviour of coal on combustion which presented some mystery. When Mr. Heron, using the micro method of the speaker and his collaborators, first communicated his results with coal, some fundamental difference between the two scales of working was sought, because it was thought that their own evidence and that of many other workers supported the view that nitrogen oxides were not formed from coal on the macro-scale or at least they were formed in negligible quantities. On the macro-scale a porcelain boat had been used, whereas Mr. Heron had used a platinum boat on the micro-scale. This seemed to be a likely cause of the differences because of the catalytic effect of the platinum. Some qualitative tests were done with coal burnt in porcelain and platinum boats, with the result that no nitrogen oxides were found in the former case, but the latter conditions showed that some formation had occurred. When the tube was packed with silica chips the formation also seemed to be enhanced slightly. Mr. Ingram carried out some similar tests at the speaker's suggestion and confirmed these findings as regards the effect of platinum and porcelain boats. An explanation was needed, therefore, as to why nitrogen oxides were formed when micro quantities of coal are burnt in the combustion tube, while the effect on the macro-scale is negligible. It might be that the same amount was formed regardless of the quantity of sample taken and that this amount, whilst significant on the micro-scale, was not apparent on the macro-scale. Against this, however, there were the very variable quantities that Mr. Heron found when using similar weights of coal. When the reason for this phenomenon had been explained we should have a clear picture of the whole process. As far as the various methods were concerned, the original instructions still held; namely, for coals on the macro-scale, nitrogen oxide formation could be ignored. On the micro-scale provision must always be made for removing nitrogen oxides when nitrogen-containing substances were present in a combustion. The only difference appeared to be that what the speaker and his collaborators had recommended as a precautionary measure, Mr. Heron has shown to be a necessity.

Mr. G. INGRAM said that with an empty combustion tube he had found that no nitrogen dioxide was produced by burning coal in a porcelain boat, but with a platinum boat some was formed. Silica chips had but little effect; only occasionally were traces of nitrogen dioxide produced. Organic substances containing nitro groups yielded traces of nitrogen dioxide when burnt in a porcelain boat; under the same conditions, those containing the amino group did not. With a platinum boat high carbon figures were obtained from amino compounds, showing that some nitrogen dioxide was formed.

The Polarographic Determination of Tin, Lead and Zinc in Phenol

BY H. N. WILSON AND W. HUTCHINSON

(Read at a meeting of the North of England Section on October 19th, 1946)

INTRODUCTION—Zinc, tin, and—occasionally—lead may be present in "pure" phenol, and methods are required for their determination. In manufacture the phenol is distilled, and a "block-tin" or tin-lined condenser is sometimes used, as it is known that phenol condensed in this way does not discolour so rapidly as that condensed in mild steel or iron. The phenol, whilst still liquid, is run into drums, which may be "galvanised," or zinc-lined. The metals are present in a few parts per million, with perhaps lead and iron also. A rapid and simple method of analysis is required.

Several variations of the dithizone reaction can be used for the colorimetric determination of zinc and lead, but these were found to be troublesome and slow; difficulties were also encountered in the colorimetric determination of tin. As polarographic methods for the determination of these elements have been described,[1,2] it was decided to attempt to devise modifications suitable for our purpose. A rapid method of sufficient accuracy was worked out. This is described below, and followed by a brief description of the experimental data on which the accuracy of the method may be assessed. The quantity of iron commonly present does not interfere, and a determination of the three metals may be completed in 3 hours. The method has now been in routine use for about eighteen months, and has proved very satisfactory.

METHOD

REAGENTS—

Hydrochloric acid, 10 per cent. w/w (sp.gr. 1·050).
Hydrobromic acid A.R. containing 46 to 48 per cent. of HBr.
Bromine A.R.
Sodium hydroxide solution, N.

Benzene, pure. Redistilled in glass apparatus.

Phenol. B.P. phenol doubly distilled in glass apparatus.

Gelatin, 0·1 per cent. solution in water.

Tin standard solution—Dissolve 1·00 g. of pure tin in a minimum of concentrated hydrochloric acid, evaporate to low bulk and make up to 1 litre with 10 per cent. (w/w) hydrochloric acid; (1 ml. ≡ 1·00 mg. of tin).

Lead standard solution—Dissolve 1·29 g. of pure lead carbonate in a minimum of dilute hydrochloric acid, evaporate to low bulk and make up to 1 litre with 10 per cent. (w/w) hydrochloric acid.

Zinc standard solution—Dissolve 1·00 g. of pure zinc in a minimum of dilute hydrochloric acid, evaporate to low bulk and make up to 1 litre with distilled water; (1 ml. ≡ 1·00 mg. of zinc.)

Prepare suitable dilutions of these standards for use as required.

PROCEDURE—

Weigh 25 g. of the phenol into a 150-ml. beaker. Add 50 ml. of benzene, warm gently until dissolved and wash into a stoppered 250-ml. separating funnel with a further 50 ml. of benzene. Extract this solution with five separate 5-ml. portions of 10 per cent. hydrochloric acid, washing each extract twice with 20 ml. of benzene and finally collect the extracts in a 25-ml. measuring cylinder. Adjust the volume to 25 ml. with 10 per cent. hydrochloric acid. It is necessary to shake vigorously and allow to settle well between washes as traces of benzene interfere with the regularity of the tin polarogram.

TIN AND LEAD—Pipette 2 or 5 ml. of the hydrochloric acid extract into a suitable polarographic cell, de-oxygenate with electrolytic hydrogen for 5 minutes, adjust the sensitivity to 1/5 and make a polarogram over the voltage range −0·2 to −0·7 volt, using the mercury pool as the anode. The half-wave potential for tin and lead occurs at −0·46 volts. Measure the step height.

LEAD AND ZINC—Pipette 10 ml. of the extract into a 30-ml. squat beaker and evaporate carefully just to dryness on a hot plate. Add 1 ml. of hydrobromic acid and two or three drops of elemental bromine, evaporate to dryness and repeat the treatment with hydrobromic acid and bromine. This treatment completely removes tin. Cool, extract the residue four times with 1 ml. of boiling *N* sodium hydroxide and combine the extracts in a 10-ml. measuring cylinder. Cool the combined extracts to room temperature, add a further 1 ml. of *N* sodium hydroxide and one drop of 0·1 per cent. gelatin solution and make up to 10 ml. with distilled water. Pipette 2 or 5 ml. of the solution into a suitable polarographic cell, de-oxygenate with electrolytic hydrogen for five minutes, adjust the sensitivity to 1/5 and make a polarogram over the voltage range −0·5 to −0·1 volt, using a saturated calomel cell as the anode. With the same photographic paper, solution and saturated calomel cell, adjust the sensitivity to 1/10, reset the zero and make a polarogram over the voltage range −1·2 to −1·8 volt. Measure the step height in both instances.

The half-wave potential for lead in *N* sodium hydroxide solution is −0·75 volt and that for zinc in the same solution is −1·50 volt.

Carry out a "blank" on the reagents and deduct the step height found for any of the metals from the corresponding step height of the particular metal polarogram. The lead and zinc contents can be read direct from graphs giving the step height in alkaline solution as concentration of the respective metals. In the case of tin, deduct the step height of the lead in alkaline solution from the combined tin and lead step height in acid solution; this gives the step height due to the tin alone, from which the tin content can then be read from the appropriate graph.

A control experiment may be run on a solution containing 0·020 g. of phenol and 0·0005 g. of tin, lead, and zinc per 25 ml. of 10 per cent. hydrochloric acid, and, if necessary, a correction applied to compensate for temperature changes. This solution is equivalent to 20 parts of tin, lead, and zinc per million, when a 25-g. sample is used for analysis.

EXPERIMENTAL

APPARATUS—The polarograph used in this work was the Cambridge instrument, the relationship between galvanometer deflection and current being 5·3 × 10^{-3} micro-amps. per division. All the polarograph work was done in a room with the temperature 20° C. ± 1·0° C. Hydrogen was used for de-oxygenating the solutions by bubbling the gas through for 5 minutes. A mercury anode was used for tin and lead in acid solution, and a saturated calomel anode for lead and zinc in alkaline solution. The capillary constant was $m^{2/3} t^{1/6} = 2{\cdot}09$ mg.$^{2/3}$ sec.$^{-\frac{1}{2}}$ at —0·45 volt in 10 per cent. hydrochloric acid (w/w), 1·88 mg.$^{2/3}$ sec.$^{-\frac{1}{2}}$ at —0·75 volt in 0·5 *N* sodium hydroxide, and 1·80 mg.$^{2/3}$ sec.$^{-\frac{1}{2}}$ at —1·5 volt in 0·5 *N* sodium hydroxide. The step height was taken as the vertical height in divisions between the points of intersection obtained by drawing straight lines through the photographic trace before, through and in line with, and after the step.

DETAIL OF EXPERIMENTAL WORK—

Much time was spent in unsuccessful attempts to find a base solution in which all three metals could be determined polarographically and, after trials of various solutions, we decided upon an acid "base" solution for the tin, and an alkaline "base" solution for the lead and zinc after removal of the tin with hydrobromic acid and bromine.

The extraction of the metals from the phenol was next investigated. As extraction is much quicker than ashing we dissolved the sample in benzene, extracted with dilute hydrochloric acid and determined the amount of the metals in the extracts. Three extractions were sufficient to remove the whole of the metallic constituents. This was proved on samples of phenol containing known amounts of tin, zinc, and lead. Pure phenol (m.p. 40·4° C.) was distilled twice in all-glass apparatus; the final material showed no trace whatever of any of the three metals when examined by the method given above.

Tin was introduced by boiling a known weight of tin foil in a weighed quantity of phenol for three hours, after which the tin foil was removed, carefully washed with benzene and methanol, dried and reweighed. It was thus ascertained that tin equivalent to 20 parts per million had dissolved in the phenol. By the method of analysis described above, duplicate tests indicated 18 and 19 parts of tin per million, a result of adequate accuracy.

Similarly, a solution of lead in phenol was prepared by boiling redistilled phenol with a known weight of bright sheet lead in exactly the same manner as with tin. This gave a figure of 30 parts per million based on loss of weight, and a polarographically determined figure of 31 parts per million.

A solution of zinc in phenol was prepared by boiling the redistilled phenol with a weighed amount of zinc dust, allowing the excess of zinc to settle out from the hot phenol, pouring off into a beaker as much phenol as possible, then recovering the zinc dust on a Gooch filter crucible, washing with methanol and finally drying at 105° C. This showed that the phenol had dissolved zinc equivalent to 75 parts per million. Analysis by the polarographic method given above showed 71 parts per million. As the zinc dust contained some zinc oxide the results are in good agreement, and as the phenol to be examined seldom contains more than 8 parts per million the accuracy is quite adequate.

It was found that small amounts of phenol had an effect on the step height of tin in 10 per cent. hydrochloric acid solution but had little, if any, effect on the step height of lead in the same acid solution. In the extraction of the phenol in benzene solution with 10 per cent. hydrochloric acid under the conditions of the analysis, approximately 0·020 g. of phenol was found in the final extract, and this amount of phenol must be added in the preparation of standards for tin. Since the lead and zinc are determined after evaporation to dryness and treatment with hydrobromic acid and bromine, phenol is absent from the solution polarographed for these metals.

In acid solution, tin and lead are reduced at the same half-wave potential of —0·45 volt against a mercury anode; therefore, in order to determine the lead, the tin must be separated from the lead either by using another base solution in which the two waves are separated and can be measured, or by removing the tin chemically. The latter method was used, as it was found extremely difficult to separate the two waves sufficiently for accurate measurement. By means of hydrobromic acid and bromine, tin (0·5 mg. in 25 ml. of the hydrochloric acid extract, ≡ 20 p.p.m. in the phenol) was completely removed in two treatments. Generally, one treatment was sufficient, but occasionally a trace of tin was left; hence we have prescribed two treatments in the final method. After removal of tin, the lead and zinc were extracted from the residue with *N* sodium hydroxide; three extractions are necessary to remove completely the whole of these metals; and the final solution is made 0·5 *N* before polarographing.

At a sensitivity of 1/10, we found that the following relationships hold. We realise that they depend on the dimensions of the capillary used, etc., but include them as a guide to other workers.

Tin in 10 per cent. HCl by weight, containing 0·02 g. of phenol per 25 ml.—
1 division of step height ≡ 0·029 mg. of tin per 25 ml.
Lead in 10 per cent. HCl by weight, containing 0·02 g. of phenol per 25 ml.—
1 division of step height ≡ 0·0415 mg. of lead per 25 ml.
Lead in 0·5 *N* sodium hydroxide solution—
1 division of step height ≡ 0·0415 mg. of lead per 25 ml.
Zinc in 0·5 *N* sodium hydroxide solution—
1 division of step height ≡ 0·015 mg. of zinc per 25 ml.

ACCURACY OF METHOD—

To establish the accuracy of the method, mixtures were made up from doubly distilled phenol and the solutions of the three metals already described, so as to contain known amounts of the metals, in about the same quantities as the samples under examination—tin about 10 parts per million, zinc less than 5 parts per million, and lead less than 1 part per million. Results are shown in the Table below.

TABLE

	Present, parts per million			Found, parts per million		
Mixture	Tin	Lead	Zinc	Tin	Lead	Zinc
1	20	nil	10	21	nil	11
2	15	5	15	14·5	4	14
3	10	10	20	11	9	21
4	5	15	nil	5	14	nil
5	10	5	15	11	4	14

The method is thus entirely adequate for this type of analysis, and compares well with any other method for traces of such metals in material of this type.

SUMMARY—

Traces of tin, lead, and zinc in phenol may be conveniently determined by the polarograph. Twenty-five g. of the phenol are dissolved in benzene and the solution is extracted with dilute hydrochloric acid. Aliquots are taken for the polarographic determination of the three metals, tin in acid solution, and lead and zinc in alkaline solution after removal of tin with hydrobromic acid and bromine. The three determinations can be completed in less than three hours.

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IMPERIAL CHEMICAL INDUSTRIES LIMITED
BILLINGHAM DIVISION
RESEARCH DEPARTMENT

Official Appointments

PUBLIC ANALYST APPOINTMENTS

NOTIFICATION of the following appointments of Public Analysts has been received from the Ministry of Health since the last record in THE ANALYST (1947, **72**, 64).

Public Analysts	*Appointments*
COOMBES, A. H. (Deputy)	City of Birmingham.
HAWKINS, Ernest Stephen	County Borough of Canterbury.
WORDSWORTH, C. H.	Borough of Luton.

The Urban District of Solihull has been constituted a Food and Drugs Area, and the U.D. Council propose to appoint Mr. F. G. D. Chalmers as their Public Analyst.

OFFICIAL AGRICULTURAL ANALYST APPOINTMENTS

NOTIFICATION of the following appointments of Official Agricultural Analysts has been received from the Ministry of Agriculture and Fisheries since the last record in THE ANALYST (1946, **71**, 586).

Official Agricultural Analysts	*Appointments*
FLINT, John Walter (Deputy)	Administrative County of Kent.
HAWKINS, Ernest Stephen	County Borough of Canterbury.
WHITTLE, Ernest George	County Borough of Bristol.

Ministry of Food

STATUTORY RULES AND ORDERS*

1947—No. 478. The Soft Drinks (Amendment) Order, 1947. Dated March 18, 1947. Pp. 2 Price 1d.

The Soft Drinks Order, 1946 (S.R. & O., 945 of 1946; ANALYST, 1946, **71**, 381) *is hereby amended* (*a*) *by modifications in the definition of "soft drinks" and* (*b*) *by providing that some soft drinks may contain tartaric or phosphoric acid.*

In the first paragraph of the definition of "soft drink" the words "suitable for or" *are inserted before* "intended for use after dilution as a drink for human consumption." *Dandelion coffee and simple and mixed herbs are now included among the products excepted from the definition.*

In Part II of the Second Schedule, dealing with the ingredients of soft drinks, the phrase "acid content" *is now replaced by* "free acid content." *As before, the acid is expressed as citric acid monohydrate, but it may now include, besides the sources of acid mentioned in the principal Order, added tartaric or phosphoric acid*; provided that no part of such acid content shall be derived from tartaric acid in the case of any description of soft drink containing citrus fruit juice, or from phosphoric acid in the case of any description of soft drink containing any fruit juice. *No change is made in the Table appended to Part II of the Second Schedule of the principal Order, except that in the heading of column* 2 *the phrase* "minimum acid content . . ." *is changed to* "minimum free acid content . . . ."

— **No. 545 The Meat Products and Canned Meat (Control and Maximum Prices) (Amendment) Order. Dated March 28, 1947.** Pp. 3. Price 1d.

Besides making various changes in maximum prices, this Order, by an insertion at the end of Article 3 *of the principal Order* (S.R. & O., No. 2046 of 1946; ANALYST, 1947, **72**, 64) *provides* that any fat of vegetable origin used in the manufacture of beef sausages, beef sausage meat, or beef slicing sausage, shall be deemed to be meat for the purpose of assessing the meat content of any of these products, if the total quantity of such fat so used does not exceed 25 per cent. of the prescribed minimum meat content of the product.

Ministry of Health

STATUTORY RULES AND ORDERS*

1947 No. 612. The Ice Cream (Heat Treatment, etc.) Regulations. Dated April 2, 1947, made by the Minister of Health under the Food and Drugs Act, 1938. Price 1d.

1. *These regulations come into operation on the first day of May,* 1947, *with the exception of* 3 (*b*) (*iv*), *which becomes operative on a date to be fixed later.*
2. In these regulations—"ice-cream" includes water ices and any article, under whatever description it is sold, which is so similar to ice-cream as to constitute a substitute therefor:

   "ingredients" includes sugar and dried egg, but does not include colouring or flavouring materials or fruit, nuts, chocolate, and other similar substances; and

   "complete cold mix" means a product which is capable of manufacture into ice-cream with the addition of water only, is sent out by the manufacturer in airtight containers, and has been made by evaporating a liquid mixture which has already been submitted to heat treatment comparable with that prescribed in these regulations.
3. The following requirements shall be observed in the manufacture of ice-cream intended for sale for human consumption:
   - (*a*) Where a complete cold mix is used which is reconstituted with wholesome drinking water and to which nothing is added other than colouring or flavouring materials, fruit,

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Obtainable from H.M. Stationery Office. Italics signify changed wording.

nuts, chocolate, or other similar substances, the reconstituted product shall be converted into ice-cream within one hour of reconstitution.

(*b*) In any other case, after the ingredients have been mixed together, the following provisions shall apply:—

(i) the mixture shall not be kept for more than one hour at any temperature which exceeds 45° F. before being subjected to heat treatment in accordance with the next following sub-paragraph;

(ii) the mixture shall be subjected to heat treatment as follows:—

It shall be raised to and kept at a temperature of not less than 150° F. for 30 minutes or alternatively of not less than 160° F. for 10 minutes;

(iii) after the mixture has been subjected to heat treatment as aforesaid it shall be reduced to a temperature of not more than 45° F. within 1½ hours and shall be kept at such a temperature until the freezing process is begun;

(iv), (v), and (vi) *the local authority is given power to specify the necessary indicating and recording thermometers and to exercise supervision over all apparatus. Any temperature charts shall be kept for at least a month.*

4. Ice-cream shall not be sold or offered for sale unless either—

(*a*) it has been kept at a temperature not exceeding 28° F. since it was frozen, or

(*b*) if its temperature has risen above 28° F. at any time since it was frozen, it has again been subjected to the treatment prescribed by sub-paragraphs (i), (ii), and (iii) of regulation 3 (*b*) and, after having again been frozen, has been kept at a temperature not exceeding 28° F.

5. Ice-cream shall be protected from dirt, dust or other contamination at all times during its manufacture, storage and distribution, and all apparatus and utensils brought into contact with ice-cream during its manufacture, storage or distribution shall be thoroughly cleansed immediately after use and shall be kept clean at all times.

CIRCULAR 69/47

This circular explaining the above regulations was issued on the 10*th April*, 1947, *by the Ministry of Health. It states that the regulations referred to in Circular* 183/46, ANALYST, 1946, **71**, 539, *have now been made* (*see above*) *and come into force, with the exception of those relating to thermometers, on the* 1*st of May*, 1947. *They define the conditions under which the ingredients of ice-cream shall be heat treated, cooled, frozen, and stored previous to sale. An exception is made for "complete cold mix" requiring the addition of no ingredient other than water, for which heat treatment is not required, but which must be frozen within an hour of its being reconstituted. Provided that all practicable steps have been taken to procure the necessary apparatus, failure to obtain it before the* 1*st of May*, 1948, *is held to be a defence to a charge of non-compliance with regulation* 3 (*b*) (*iii*). *The date from which the indicating and recording thermometers considered necessary by local authorities are to be put into compulsory use remains to be announced by the Minister; but the charts of any recording instruments in use are to be kept for at least a month,* even though the compulsory requirement of such thermometers has not come into operation. *The general cleanliness of all materials and apparatus, together with the operation of the plant, is subject to supervision by the local authority.* The Minister has given further consideration to the prescription in the Regulations of a bacteriological standard of cleanliness for ice-cream, but he is still not satisfied that there is any test, the reliability of which is sufficiently established to justify its use as a statutory test, non-compliance with which would constitute an offence. He desires, however, to draw attention to a form of methylene blue test, adapted for testing ice-cream, particulars of which have been published in the Ministry of Health's Monthly Bulletin for March, 1947. The Bulletin is sent to Medical Officers of Health. Application for these particulars by other persons interested should be made to the Secretary, Ministry of Health, Whitehall, S.W.1. As pointed out in the Bulletin, the conclusions and suggested grading are at present provisional, but the Minister is advised that this test of bacterial cleanliness appears to provide the best available for the present purpose. At the same time it is simple and cheap to perform. It is suggested that if, out of the four grades recommended, ice-cream consistently fails to reach grades one and two, it would be reasonable to regard this as indicating defects of manufacture or of handling which call for further investigation. The Minister has also had regard to the representations which have been made to him on a number of other points with a view to strengthening the Regulations, particularly in relation to the protection of ice-cream after manufacture. While, however, he fully appreciates the importance of the latter, he has come to the conclusion that the Regulations as now made represent as much as it is at present practicable to require.

April 10, 1947

The Dysonian Notation

THE following account of the Dyson system for the nomenclature of organic compounds has been condensed from the record of the lecture delivered by G. Malcolm Dyson, M.A., Ph.D., M.I.Chem.E., F.Inst.Pet., F.R.I.C., on October 21st, 1946, under the joint auspices of the Chemical Society, the Royal Institute of Chemistry, the Society of Chemical Industry, and the Bureau of Abstracts, and published by the Royal Institute of Chemistry.*

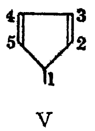
The rules governing the system are here given in the form of instructions for expressing the structural formula or ideograph of an organic compound as a CIPHER, or line of capital letters, numbers, stops, and symbols.

Describe each feature of the formula in turn as a separate OPERATION, dividing operations from each other by STOPS. Number every carbon atom in order as it appears in the ideograph, progressing first through the longest straight chain, following outwards from this along the next longest chain and proceeding in turn along the remaining branches in decreasing order of length. If *polyfunctional groups* do not occur, *i.e.*, groups having an acyclic carbon chain broken by non-carbon atoms as in esters, ethers, etc., start each section of the cipher with a description of the *basic carbon skeleton.* If this is *acyclic,* cipher it in terms of the longest carbon chain present, using C to denote the chain, followed by a MODULANT or number corresponding to the number of carbon atoms in the chain (if the modulant is 1, omit it). Thus, methane is C, ethane is C2, octane is C8. Cipher branched chains in terms of the longest straight chain, adding the branches in subsequent operations, the longest first. Start the operation describing a branch chain with a LOCANT, a number showing to which carbon atom of the main chain the branch is attached. Follow the locant with a description of the branch modulated similarly to that of the main chain; thus, *iso*-butane is C3.2C. Make all additions to the main chain in order of decreasing size. Group together in a single operation two or more similar fragments, separating their locants by a COMMA; thus 2,2,4C indicates three methyl groups in the 2, 2 and 4 positions. Cite a series of locants forming an arithmetical series of four or more terms in the form *a*(*b*)*c*, where *a* and *c* define the limiting carbon atoms of the series and *b* defines the constant difference; thus, one writes 4(2)10C and not 4,6,8,10C.

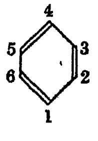
Denote *unsaturation* by the letter E, modulated thus: E, ordinary double bond; E1, double bond with *cis-* arrangement of groups; E2, double bond with *trans-* arrangement; E3, triple bond. Ethylene is C2.E; butadiene is C4.1,3E; *cis*-butene is C4.2E1. Whatever the unsaturation, the initial operation must delineate the longest carbon chain. Cite E operations in order of their modulants, *e.g.*, E before E3.

Cipher *saturated cyclic hydrocarbons* by the letter A, which shows that a ring has been created; thus, AC3 is *cyclo*-propane, AC6 is *cyclo*-hexane. Show a second or third ring as a subsequent operation, indicating the two carbon atoms of the main ring between which the subsidiary ring lies by HYPHENED locants; thus, thujane is AC6.1–3A.C2.4–7C. Indicate carbon atoms in the subsidiary rings in the appropriate A operation; thus, adamantane is AC8.1–5,2–7AC, since each subsidiary ring contains a CH_2 group.

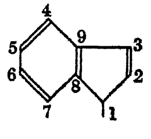
Relate all *aromatic rings* to the six conventional rings set out below with the letters used for them.



V



B



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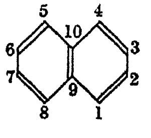
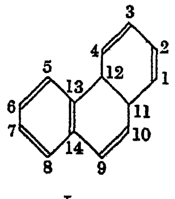
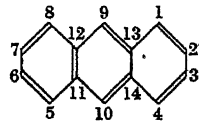


K



J



Thus, toluene is B.C.; *p*-xylene is B.1,4C; 5-methyl-4-ethyl-1-butyl-phenanthrene is J.C4.4C2.5C. Signify the extra hydrogen with V and W by a modulant as in V1, W1, W2. Cite rings in the same structure in this order of seniority: T, J, K, W, B, V, AC; enclose subsidiary rings in SQUARE BRACKETS, beginning enumeration afresh for each symbol so enclosed. Note that the locants of the conventional rings are numbered anti-clockwise, except in T, where the traditional enumeration is preserved.

Indicate *partial hydrogenation* of a ring by the letter H; thus, dihydrophenanthrene is J.9,10H. Place H before a ring symbol to indicate *complete hydrogenation* as in HT, tetradecahydroanthracene. *Intermediate hydrogenated rings* may be shown by inserting E operations into the fully hydrogenated ring or by a separate operation in H conjoined with the aromatic ring, using whichever leads to the shorter cipher.

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* A fuller account of the system will be found in the book by Dr. Dyson, reviewed on p. 176.

Use the following modulated forms of T, J, K, and W to delineate the more commonly encountered formulae:

Cipher	Extended cipher	Cipher	Extended cipher
T1	T.1–2B4	J3	J.9–10B4
T2	T.2–3B4	J4	J.1–2,3–4B4
T3	T.1–2,3–4B4	J5	J.1–2,5–6B4
T4	T.1–2,5–6B4	J6	J.1–2,7–8B4
T5	T.1–2,6–7B4	J7	J.3–4,5–6B4
T6	T.1–2,7–8B4	J8	J.1–2B3
T7	T.2–3,6–7B4		
T8	T.1–2,15–16B4	K1	K.1–2B3
T9	T.1–2,16–17B4	K2	K.2–3B3
T10	T.1–2,17–18B4	K3	K.1–8B3
		K4	K.1–8B2
J1	J.1–2B4		
J2	J.3–4B4	W1/1	W1.2–3B4

Enumerate carbon atoms of subsidiary rings in ascending order as one proceeds round the ring from the carbon atom of the main ring having the lower locant to that having the higher. Number the carbon atoms of the subsidiary rings in T1 to T10 and J1 to J7 inclusive in this manner: for the subsidiary ring first mentioned in the cipher, 15, 16, 17, 18; for the second, 19, 20, 21, 22. Number those in the subsidiary ring of J8 similarly, 15, 16, 17; those of that in K1, K2, K3, 11, 12, 13; of that in K4, 11, 12; of that in W1/1, 10, 11, 12, 13.

Start the cipher of a *fused ring* from its most senior constituent ring. If it comprises elements of structure over and above those of the six conventional rings, build up its cipher by adduction; thus, J.4–5AC indicates that annellation has taken place at the 4–5 position, and that the adduct is a methylene group. Adducts may be saturated as in AC, AC2; or unsaturated portions of the benzene ring as in B1, B2, B3; odd numbered B adducts need an additional hydrogen citation to complete the structure.

If a hydrocarbon comprises two or more identical rings joined either directly or through a single carbon atom, θ is used to show duplication, 3θ triplication, 4θ quadruplication and so on. θ may be used indefinitely in handling functional links but only once in citing a hydrocarbon structure. Delineate hydrocarbons in which several rings are joined to a chain of carbon atoms or to another and different ring by citing first the chain or ring and treating the several rings as substituents.

Two types of *heterocyclic compound* are recognised. In the first, adducts to hetero-derivatives of conventional rings are more than half carbon; in the second the adducts are more than half heterogeneous. Cipher the first type by adding operations in ZQ (*hetero-oxygen*), ZS (*hetero-sulphur*), ZN (*hetero-nitrogen*), etc., after citation of the corresponding homogeneous structure; thus, pyridine is B.ZN. Cite in this order: ZQ, ZS, ZSE (selenium), ZTE (tellurium), ZN, ZP, ZAS (arsenic), ZSB (antimony), ZBI (bismuth), using capital letters for the hetero-elements. Cipher the second type in a sequential operation as in B.1–2AQCQ, methylenedioxy-benzene.

Use the following modulated forms of N to denote nitrogenous fractions: N, *amino*; N1, *nitroso*; N2, *nitro*; N3, *azido*; N4, *azo*; N5, *pentacovalent nitrogen*; N6, *alidiazo*.

Cite *functional groups* with Q to denote *oxygen* and *hydroxyl*, EQ for *carbonyl*, X for *carboxyl*; thus, ethanol is C2.Q; acetaldehyde is C2.EQ; acetic acid is C2.X; acetone is C3.2EQ. This suffices for all *alcohols*, *glycols*, *aldehydes*, *ketones*, and *acids*, and compounds containing any or all such groups together.

Cipher simple *ethers* and *esters* from the side containing the larger hydrocarbon residue; thus $CH_3CH_2OCH_2CH_3$ is C2.Q[C2]; $CH_3CH_2COOC_2H_5$ is C3.X[C2]; $C_6H_5{-}OCH_2CH{=}CH_2$ is B.Q[3C3.E]; $CH_3COOC_3H_7$ is C3.[XC2]. If an ester is ciphered from the acid side, cite X outside a SQUARE BRACKET containing the cipher of the alcoholic residue; if from the alcohol side, cite X within a SQUARE BRACKET containing that of the acid residue. Cipher esters of polyhydric alcohols from the alcohol side, and esters of polycarboxy acids from the acid side; thus tripalmitin is C3.1,2,3[XC16]; tri-ethyl tricarballylate is C5.3C.1,5,6X[C2].

If the compound is *polyfunctional*, containing a non-cyclic carbon chain broken by non-carbon atoms other than nitrogen in amines, deal first with that portion showing the largest number of operative polyfunctions; if two such portions are equal in length, choose that with the larger number of potential poly-

1 2 3 4

functions to start with. Thus, in ciphering $C_2H_5O.CO\ CH.CH_2OH\ CH_2O\ CO\ CH_2\ CH_2\ COOH$ start with (1),

(1)

a portion having two operative and one potential polyfunctions: C4.X[C2].4[XC4.4X].3Q. Again, glyceryl α-succino-dipalmitate is C3.[X.C4.4X].2,3,[XC16], because (*a*) glycerol has three operative functions and (*b*) the succinic acid has one operative and one potential polyfunction. K. A. W.

OFFICIAL AND TENTATIVE METHODS OF THE AMERICAN OIL CHEMISTS' SOCIETY*

THE methods, bound in a loose-leaf binder, are divided into nine sections and are as follows:—

Section A: Vegetable Source Materials—Sampling of cottonseed, peanuts and soyabeans; determinations of moisture and volatile matter, oil, nitrogen-protein-ammonia, free fatty acids in these three materials; determination of residual lint in cottonseed.

Section B: Oilseed By-Products—Sampling of cake, meal, meats, linters, hull fibre; determination of oil content of these materials; determination of moisture and volatile matter and nitrogen-protein-ammonia in cake, meals, meats; determination of cellulose yield in linters, hull fibre.

Section C: Commercial Fats and Oils—Sampling; determination of moisture by distillation, hot plate, air oven, and vacuum oven methods; determination of soluble mineral matter and fatty acids combined as mineral soap, of free fatty acids, of unsaponifiable matter in marine and other oils; detection of chlorinated solvents (Beilstein test), of sulphur (coin test) (silver benzoate test); determination of refining loss for extracted and de-gummed soyabean oils and for other oils; break test; Halphen test; detection of sesame and teaseed oils; Crismer test; detection of foreign fats in pork fat; determination of melting point by capillary tube and Wiley methods, of softening point and slipping point; flow test; cloud test; determination of refractive index; bleaching tests for cottonseed oil, soyabean oil and animal fats; determination of smoke, flash and fire points; determination of specific gravity of oils and liquid fats, of solid fats and waxes; cold test, titre test; determination of colour, by F.A.C. and Wesson methods, of iodine value, of saponification value, of thiocyanogen value, of acetyl and hydroxyl values, of Reichert–Meissl, Polenske, Kirschner values, and of liquid and solid fatty acids.

Section D: Soap and Soap Products—Sampling; determination of moisture and volatile matter by air oven method, of moisture by distillation, of alcohol-soluble and alcohol-insoluble matter, of free acid or alkali in soda soaps, of free alkali or carbonate in potash paste soaps, of water-insoluble matter, of the total alkalinity of alcohol-insoluble matter, of total anhydrous soap and combined alkali, of total anhydrous soap in the presence of synthetic detergents, of chlorides, of unsaponified plus unsaponifiable matter, of unsaponifiable matter, of rosin. Titre test; determination of acid value of fatty acids, of iodine value, of saponification value, of borax, of alkaline silicates, of carbonates by gravimetric absorption method, and by volumetric evolution method, of phosphates, of tetrasodium pyrophosphate, of sulphates, of glycerol, of sugars, of starch, of volatile hydrocarbons, of combined sodium and potassium oxides; screen test; determination of fatty matter in soaps containing synthetic detergents.

Section E: Glycerin—Sampling; determination of ash, of alkalinity or acidity, of sodium chloride, of total and organic residue, of glycerol by acetin and dichromate methods.

Section F: Sulphonated and Sulphated Oils—Determination of moisture by distillation, of moisture and volatile matter by hot plate method, of organically-combined sulphuric anhydride by titration, extraction-titration and ash-gravimetric methods, of total de-sulphated fatty matter, of active ingredients, of unsaponifiable non-volatile matter, of inorganic salts, of total alkalinity, of total ammonia, of acidity in the presence of ammonium or triethanolamine soaps and in their absence, of acidity in dark coloured oils, of water-immiscible solvents.

Section G: Soap Stock—Sampling; determination of total fatty acids by wet and dry extraction methods; of total fatty acids in coconut oil soap stock; of neutral oil; titre test.

Section H: Specifications—Forced draught oven; petroleum ether; air oven; vacuum oven; smoke point thermometer; thermometers for titre test, Crismer test, flow test; desiccants.

Section I: Tables—Tables are presented showing the physical and chemical characteristics of 109 different kinds of fats, oils and waxes, and of 14 authentic samples of cottonseed oil.

All the methods above are described as Official except that for determining the refining loss from extracted soyabean oils, which is a Tentative method, and that for the cellulose yield of cottonseed linters and hull fibre, which is neither Official nor Tentative.

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ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Determination of Egg Yolk in Egg White. J. H. Cook and V. C. Mehlenbacher (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 785–788)—As little as 0·05 per cent. of yolk in egg white has an adverse effect upon its baking qualities. Owing to difficulties in the extraction of small amounts of fat, estimation of yolk contamination by determination of fat proved unsatisfactory, and the possibility of determining the cholesterol carried into the white by the yolk was investigated. For this purpose the colour development in the Liebermann-Burchard reaction was studied. Five ml. of a solution in chloroform of pure cholesterol were treated with 2 ml. of a 10 to 1 mixture of acetic anhydride and sulphuric acid, and the mixture was placed in the dark for 24 min. at 35° C. The resulting green colour was measured in a Coleman Universal Model 11 spectrophotometer against the colour of pure chloroform in the solvent cell. The transmittance was determined over the range 400 to 800 mμ with a PC-4 filter. The point of maximum absorption, *viz.*, 640 mμ, was used in all subsequent measurements. The intensity of the colour was found to depend not only upon the concentration of cholesterol but also upon the temperature and the time of standing. Ireland (*Biochem. J.*, 1941, **35**, 283; ANALYST, 1941, **66**, 345) showed that the use of lower temperatures during colour development leads to better results, and it was found that colour development at 18° C. in the dark gave the best results with the necessary stability at the point of maximum absorption. The change in transmittance between readings taken at 20- and 30-min. intervals is 0·5 per cent., and, consequently, it is not necessary to make the measurements after the lapse of an exact period of time. In applying the Mojonnier modification of the Röse-Gottlieb method to the extraction of cholesterol it was found necessary to add 25 ml. of aqueous ammonia to the sample before adding any of the extracting solvents; otherwise the alcohol coagulates the albumin to a gelatinous mass that resists extraction. In determining the cholesterol in egg white a 10-g. sample is required,but with yolk a 1-g. sample is used, the extracted cholesterol being dissolved in 250 ml. of chloroform and a 5-ml. aliquot taken. In numerous individual yolks the amount of cholesterol ranged from 1·2 to 1·4 per cent. with an average value of 1·32, and this agrees with reports in the literature. Eggs with varying histories were separated and the whites, uncontaminated with yolk, were analysed to determine the blank value obtained when no yolk was present. This value appears to be constant for all egg whites, ranging only from 0·00011 to 0·00012 (average 0·000114) g. of cholesterol per 10 g. of egg white. Mixtures of yolk and white in varying amounts were then made and analysed.

Method—To prepare the reagent, add 10 ml. of sulphuric acid (sp.gr. 1·84) slowly and with cooling to 100 ml. of freshly distilled acetic anhydride in a 200-ml., glass-stoppered flask. The reagent should be clear and colourless. To prepare the standard curve, dissolve 50 ± 0·2 mg. of pure cholesterol in 500 ml. of chloroform and pipette 1, 2, 3, 4, and 5 ml. of the solution into test tubes (15 × 150 mm.), and make the total volume in each tube 5 ml. by addition of chloroform. Place the tubes in a light-proof water-bath maintained at a constant temperature of 18° C. for 5 min. Add 2 ml. of the acetic anhydride and sulphuric acid mixture, also at 18° C., to each tube, mix thoroughly with a glass rod, and allow the tubes to stand for 25 min. at 18° C. Determine the transmittance in a spectrophotometer at 640 mμ with a solvent cell of pure chloroform set at 100 per cent. transmittance. Plot transmittance (logarithmic ordinate) against concentration on semi-logarithmic paper, thus establishing the standard curve.

To 10 ± 0·1 g. of liquid egg white in a 200-ml., glass-stoppered flask, add 25 ml. of aqueous ammonia (sp.gr. 0·90), swirl the mixture, add 10 ml. of 95 per cent. alcohol, and shake for 1 min. Add successively 25 ml. of ethyl ether (A.C.S. specification) and 10 ml. of light petroleum (b.p. 35° to 38° C.), shaking for 1 min. after each addition, and allow the mixture to stand until the upper layer is clear. Break emulsions, if necessary, with 5 ml. of alcohol. Decant the clear solvent layer into a 250-ml. beaker, repeat the extraction of the residue twice, beginning with the addition of alcohol but using 5 ml. of alcohol each time and adding the final solvent layers to the original extract in the beaker. Evaporate the extract on the water-bath until only a small amount of alcohol remains, cool, add 15 ml. of alcohol, boil on the hot plate, add 1 ml. of 50 per cent. aqueous potassium hydroxide solution, and boil gently for 10 min. Cool, rinse down the sides of the beaker with 30 ml. of ether, and transfer the liquid into a 250-ml. separating funnel. Rinse out the beaker with two 25-ml. portions of water and transfer each into the separating funnel. Rotate the funnel without shaking, break any emulsion that forms with 5 ml. of alcohol, withdraw the lower soap layer, and wash the ether layer with 50-ml. portions of water three times or until neutral to phenolphthalein. Evaporate the ether layer to complete dryness on a water-bath, cool, and pipette 5 ml. of chloroform into the beaker, swirling the mixture gently to ensure solution. Transfer the solution into a test tube (15 × 150 mm.) and place it in the light-proof water-bath maintained at 18° C. for 5 min. Proceed as in the construction of the standard curve and read the transmittance of each solution. From the standard curve find the concentration of cholesterol corresponding to the transmittance. A blank determination should be made by evaporating to dryness a mixture of all the reagents used, and subjecting the residue to the whole process of analysis; the value so obtained should be deducted from that obtained with the sample. A table is provided for conversion of cholesterol found into percentage of egg yolk in the egg white. The tabular values do not differ widely from the figures obtained by deducting the apparent cholesterol content of egg white (0·00011 g. per 10 g.) from the determined content of the sample and calculating the amount of yolk on the basis of 1·32 per cent. of cholesterol in egg yolk.

A simple, constant-temperature water-bath can be made by wrapping several sheets of asbestos paper round a suitable tin can, lining the lid with asbestos, and piercing it to form a hole only just large enough to take a thermometer. A. O. J.

Semi-micro Determination of Calcium in Foodstuffs. L. C. E. Kniphorst (*Chem. Weekblad*, 1946, **42**, 54–59, 66–70)—The chief difficulties in the determination of calcium in foodstuffs result from contamination of the calcium oxalate precipitate by magnesium, interference by phosphates or pyrophosphates at a *p*H of about 5, and delayed or incomplete precipitation of calcium oxalate under certain conditions. The method is applied to an ash containing 5 to 12 mg. of calcium oxide, not more than 90 mg. of magnesium oxide or 400 mg. of phosphorus pentoxide, and, at most, 5 mg. of iron + aluminium + manganese + copper.

Procedure—Dissolve the ash by repeated extraction with hot 4 *N* hydrochloric acid (in all 35 ml.), re-ignite any carbonaceous residue, and extract again. Dilute the filtered solution to 100 ml., heat to 100° C., and keep on the water-bath for one hour to decompose pyrophosphate. After the addition of bromocresol purple, neutralise the liquid by means of aqueous ammonia, acidify to a yellow colour with acetic acid, and dilute to 200 ml. Heat to boiling, remove the flame, and add 10 ml. of boiling ammonium oxalate solution (saturated at ordinary temperature), with stirring, to the clear liquid. If, however, the liquid is not clear, owing to the presence of phosphates, add bromocresol green, followed by just sufficient 4 *N* hydrochloric acid to clear it. Add then the hot ammonium oxalate solution, followed by 60 per cent. ammonium acetate solution added drop by drop until the *p*H is about 5 (a definite blue colour). Small quantities of iron, aluminium, copper, zinc, and manganese are held in solution by the excess of ammonium oxalate. The quantities of acid, ammonia, and ammonium acetate added must be kept at a minimum, since they are liable to delay the precipitation of the calcium. In general, 5 ml. of ammonium acetate should be sufficient; 8 ml. is the maximum permissible. Keep the mixture on the water-bath for four hours, and stir and scratch with a glass rod at intervals during the first 30 min. If the precipitate appears within ten minutes, filtration may be carried out after four hours; but otherwise the liquid should be left overnight, and then filtered cold. Wash the precipitate twice with a little cold water, then about six times with saturated calcium oxalate solution, until the filtrate shows only a weak reaction for chloride ion. Transfer the precipitate to a beaker, and wash the filter with 25 ml. of hot 4 *N* sulphuric acid. Dilute to 250 ml. with boiling water, treat with 15 ml. of sulphuric acid (1+1), and titrate with 0·05 *N* potassium permanganate added from a micro-burette. Carry out a blank titration with the same quantity of water and sulphuric acid. The maximum error of this method is 5 per cent.

An alternative method, which gives greater precision, is to ignite the precipitated calcium oxalate to lime, which is then dissolved in 10 ml. of hot, 10 per cent. boric acid solution, diluted with 50 ml. of water, and titrated with 0·05 *N* hydrochloric acid. The indicator is methyl red - methylene blue (Patterson, *Biochem. J.*, 1925, **19**, 601) or methyl red - bromocresol green (Reith and Klazinga, *Chem. Weekblad*, 1941, p. 122), and the end-point is observed by comparison with a solution containing 10 ml. of boric acid solution diluted to 65 ml., and indicator. G. M.

Source of Error in the Gutzeit Method for Arsenic. N. I. Goldstone (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 797–799)—Suggested modifications of the official and empirical Gutzeit method have not been widely adopted since they involve either special apparatus or complex procedure. The official method specifies standards of reagents, apparatus, and procedure, but leaves the form of the hydrogen-generating zinc undefined. Stick zinc can be selected only by a visual comparison of the rates of gas evolution, whilst granular zinc does not produce a constant stream of gas. The use of a pellet, the constant surface area of which would produce a uniform rate of gas evolution, thus increasing reproducibility of length and intensity of stain, is suggested.

Method. Preparation of pellets—Select a number of Pyrex tubes of identical internal diameter (approximately 15 mm.) and heat them carefully in a vertical position. Fill the test tubes with molten, arsenic-free zinc and, after tapping out air bubbles, allow to cool slowly. The upper portion should be maintained liquid until last, thus preventing the formation of a hollow core. Break away the tubes, and cut the rods into suitable lengths, the ends being smoothed with emery. Cover the smooth ends with a paste of magnesium carbonate in gum arabic and allow to dry. Dip the pellet into a suitable wax to give a coat about 0·16 cm. thick. Scrape the ends free of wax and soak off the paste. Activate the pellets with a solution of diluted stannous chloride - hydrochloric acid solution (1+7) and store under water containing a drop of hydrochloric acid. A pellet 3·75 cm. long can be used for about 15 determinations.

Sensitisation of paper strips—Cut a sheet of 32 Hanford-Pratt paper strips into 9-cm. lengths and suspend them in alcoholic mercuric bromide solution in a 10-ml., glass-stoppered graduated cylinder in the dark.

Reagents—Solutions containing 0·002 mg. of arsenic per ml., 0·005 mg. of arsenious oxide and 5 ml. of hydrochloric acid in 35 ml., and 0·10 mg. of arsenious oxide and 5 ml. of hydrochloric acid in 35 ml. were used to test the pellets. A solution of digested oysters was prepared and acidified to the extent of 5 ml. of hydrochloric acid in 35 ml.

Alcoholic mercuric bromide. A 4 per cent. solution in 95 per cent. ethanol.

Potassium iodide. A 15 per cent. aqueous solution.

Stannous chloride. A 40 per cent. solution of the dihydrate in concentrated hydrochloric acid.

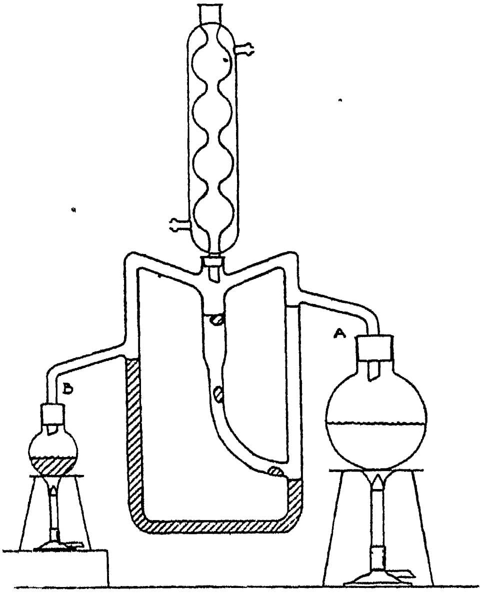
Clean sand moistened with 10 per cent. lead acetate was used in the absorption tower to remove hydrogen sulphide.

Procedure—Sets of six determinations on each solution were made on different days. 35-ml. portions were transferred to generating bottles and 5 ml. of potassium iodide solution added, then 4 drops of stannous chloride solution, and the solutions were left to stand for 0·5 hr. at 25° C. The zinc pellets were then added, the absorption tubes connected, and the generators submerged for 1·5 hr. in a bath at 25° C. The strips were then removed and the stains measured by the usual method.

Discussion—The pellets gave an accuracy comparable with that of the most carefully controlled conditions used by How (*Ibid.*, 1938, **10**, 226) using a zinc alloy and sensitised cotton threads, *i.e.*, 3 per cent. on a single determination. No previous attempt has been made to standardise the exposed area or the uniformity of gas evolution. The pellet surface becomes coated with tin, but the uniformity of rate of evolution of gas is apparently not affected. The pellets wear evenly without pitting of the surface, and there appeared to be no difference between the activities of the six pellets used during the experiments. A combination of the constant area pellet with sensitised cotton threads would probably greatly increase the accuracy of arsenic determinations in the range of 1 µg. of arsenious oxide.

M. E. D.

Titrimetric Determination of Di-acidic Bases (Nicotine, Quinine) in Mixtures with Mono-acidic Bases, and a Simplified Assay of Nicotine. E. M. Trautner and O. E. Neufeld (*Australian Chem. Inst. J.*, 1946, **13**, 70–74)—Of the numerous methods available for the determination of nicotine in the presence of lower amines, which are often liberated from the drug during the assay procedure, it is stated that the simple ones are unreliable, while the reliable ones are complicated and time-consuming. Advantage is usually taken of the fact that the nicotine bases, like the cinchona bases, can be titrated as mono-acidic or di-acidic depending on the conditions. In chloroform solution, the cinchona bases can be titrated accurately as di-acidic bases with *p*-toluene sulphonic acid solution, dimethyl yellow being used as indicator. In aqueous-alcoholic solution, they react as mono-acidic bases to mineral acids when methyl red is used as indicator. Thus, the difference between the titration figures corresponds to the second nitrogen of the di-acidic bases, while twice the aqueous-alcoholic titration figure, less that found for the chloroform solution, is equivalent to the total nitrogen of all the bases present that have not altered their titration values in the two solvents. Nicotine in solutions in chloroform cannot be titrated satisfactorily with *p*-toluene sulphonic acid, but 0·05 *N* picric acid with dimethyl yellow as indicator gives accurate figures corresponding to a di-acidic base. The solution should be warmed to about 40° C. to prevent precipitation of the monopicrate as it is formed; precipitation of the dipicrate in no way interferes with the accuracy of the titration. Tested on synthetic mixtures of nicotine with ammonia and with methylamine, the method



gives an accurate indication of the amount of diacidic base present.

The determination of nicotine in tobacco, etc., can be rapidly carried out by extracting the drug by distillation in the apparatus shown. *In the illustration the condenser and the flasks are drawn to half the scale of the separator.* The inner diameter of the separator tubes is 7 to 8 mm. The upper side arm, *A*, is connected to a flask containing the sample under examination immersed in aqueous alkali, and chloroform is placed in the other flask. The contents of both vessels are kept gently boiling, and the mixed vapours are condensed and separated, each being returned to its respective flask. The transfer of the nicotine to the chloroform proceeds smoothly and quickly. *Procedure for tobacco*—Transfer to the water flask such an amount of the drug or the extract, powder, etc., as might be expected to contain between 0·1 and 1·0 g. of nicotine, add 100 ml. of water, 3 g. of sodium hydroxide, and 20 to 30 g. of sodium chloride. A small amount of paraffin wax may be added to inhibit frothing. Charge the other flask with 50 to 75 ml. of chloroform, and heat the contents of both flasks to boiling. After the heating has continued for 1·5 to 2 hr., filter the chloroform through a dry filter, dilute to a known volume, and titrate an aliquot portion with 0·05 *N* picric acid.

With most samples of drug, separation from the volatile bases is sufficiently complete for the aqueous titration to be omitted. The procedure has been checked against that employing precipitation of nicotine silicotungstate, and close agreement between the two methods is indicated. J. A.

Biochemical

Estimation of Vitamin A and Carotene in Small Quantities of Blood Serum. O. A. Bessey, O. H. Lowry, M. J. Brock, and J. A. Lopez (*J. Biol. Chem.*, 1946, **166**, 177–188)—In this method the serum is saponified and extracted on a micro-scale with solvents of low volatility. The light absorption at 328 and 460 mμ is measured, the vitamin A absorption at 328 mμ is then destroyed by irradiation, and the absorption at 328 mμ is again measured.

Method—Into test tubes, 10 cm. × 3 mm., put 60 c.mm. of serum and 60 c.mm. of *N* potassium hydroxide in 90 per cent. ethanol (freshly prepared), immerse the tubes in a water-bath at 60° C. for 20 min., cool, and add 60 c.mm. of a 1:1 mixture of kerosene and xylene. Mix the contents of the tube by holding the tubes at an angle of 45° against a rapidly rotating metal rod slightly flattened at one point, and then centrifuge for 10 min. at 3,000 r.p.m. Cut the tube with a file just above the kerosene-xylene layer and transfer this by means of a pipette into the micro-cuvette of a Beckman spectrophotometer. Measure the absorption at 460 and 328 mμ, transfer the sample to a tube, 4 cm. × 2·5 mm., and irradiate by means of a mercury vapour lamp for 6 or 8 times the time necessary to destroy 50 per cent. of the vitamin A present, as determined by previous experiments with solutions of known vitamin A content. Take a second reading at 328 mμ. In order to eliminate interference due to turbidity, rinse with anhydrous propionic acid, for 1/3 to 1/2 of its length below the constriction, the pipette used to transfer the sample back to the cuvette after irradiation.

Calculation—Calculate the amount of carotene present (in μg. per 100 ml.) by multiplying the absorption at 460 mμ by 480, and the amount of vitamin A (in μg. per 100 ml.) by subtracting the second reading at 328 mμ from the first, and multiplying the difference by 637. The factor 637 is based on a value of 1720 for $E^{1\%}_{1\,cm.}$ at 328 mμ of vitamin A palmitate in alcohol, calculated as the free alcohol. Since vitamin A ester has only 96 per cent. of this value in kerosene-xylene and still retains 3 per cent. of its initial absorption after irradiation, and since furthermore the absorption is reduced 2 per cent. owing to the use of a wide spectral band, the nett value of $E^{1\%}_{1\,cm.}$ is

$$1720 \times 0{\cdot}96 \times 0{\cdot}97 \times 0{\cdot}98 = 1570$$

and

$$1{,}000{,}000/1570 = 637.$$

The method gave essentially the same values for carotene and vitamin A as did a macro-procedure using the Carr-Price reagent. F. A. R.

Tocopherol Content of Skeletal Muscle: Comparison of Chemical and Bio-assay Methods. H. Kaunitz and J. J. Beaver (*J. Biol. Chem.*, 1946, **166**, 205–217)—*Procedure*—Emulsify 30 to 50 g. of muscle with about 100 ml. of acetone in a Waring blendor for 6 min. and transfer to a centrifuge tube with an additional 350 ml. of acetone. After 24 hours, centrifuge, extract the residue once more with acetone, then once with a 1:1 mixture of acetone and light petroleum, and finally with a 1:2 mixture of acetone and light petroleum. Transfer the extracts to a 3-litre separating funnel and add water until 2 layers are formed. Remove the petroleum layer and extract the aqueous phase with two 200-ml. portions of petroleum. Wash the combined petroleum extracts with three 500-ml. portions of water, centrifuge, and evaporate in an all-glass apparatus under reduced pressure in an atmosphere of nitrogen. Transfer the residue to a 25-ml. flask with about 20 ml. of light petroleum. Add 1 to 2 ml. of sesame oil and make up to 25 ml. with petroleum. (The addition of an oil reduces the time required for development of the maximum colour. Sesame oil was selected because it contained only negligible amounts of reducing substances: 0·1 ml. dissolved in 2 ml. of light petroleum should give an extinction value of less than 0·002 when mixed with 10 ml. of the iron-dipyridyl reagent). The mixture should be slightly yellow and quite clear. To estimate the carotene present, evaluate the colour in a spectrophotometer, with light petroleum as blank, at 440 mμ, and calculate the carotene concentration from a calibration curve.

To estimate the tocopherol content, pipette 1-ml. aliquots of the muscle extract into 3 test tubes, and to one add 1 ml. of light petroleum, to another add 1 ml. of a standard solution containing 40 to 50 μg. of synthetic *dl*-α-tocopherol per ml. of light petroleum, and to the third add 1 ml. of a standard solution containing 80 to 100 μg. per ml. Next add to each of the 3 tubes, 10 ml. of iron-dipyridyl reagent (250 mg. of $FeCl_3.6H_2O$ and 500 mg. of $\alpha\alpha'$-dipyridyl in 1 litre of glacial acetic acid). Use as a blank 2 ml. of light petroleum containing the same amount as the unknown of the same sesame oil plus 10 ml. of the iron-dipyridyl reagent. Keep the solutions in the dark and evaluate the colours every minute until a maximum reading is obtained. The dilutions of the extract should be such that none of the readings exceeds 0·4. Calculate the results (μg. per ml.) from the expression $e_1n/(e_2-e_1)$ where e_1 is the value obtained for the unknown, e_2 is the value obtained for the unknown plus n μg. of tocopherol. For each μg. of carotene present per ml., subtract 4·8 from the result so obtained. With different amounts of tocopherol and different concentrations of extract, the maximum error observed did not exceed ± 10 per cent. The results were compared with those obtained by bio-assay, and the agreement was within ± 10 per cent.

F. A. R.

Rapid Photocolorimetric Micro-procedure for Blood Sugar. B. D. Polis and M. Sortwell (*Arch. Biochem.*, 1946, **11**, 229–233)—The use of perchloric acid as a protein precipitant gives a convenient method of producing protein-free filtrates having, when mixed with alkaline copper reagent, a *p*H that renders them insensitive to impurities. A modified phosphomolybdic acid reagent has been devised that gives a blue colour stable over a period of 24 hours.

Method—Pipette 0·1 ml. of whole blood into 4·9 ml. of 3 per cent. perchloric acid, shake the mixture, and filter or centrifuge. Put 1 ml. of the clear filtrate into a Folin-Wu sugar tube and add 1 ml. of water and 2 ml. of copper reagent. (To prepare this reagent, dissolve 140 g. of anhydrous sodium carbonate in 800 ml. of water, add 52 g. of sodium tartrate and 44 g. of sodium bicarbonate. Dilute to about 3 litres and shake until the solid is dissolved. Dissolve 20 g. of $CuSO_4.5H_2O$ in a small amount of water, add the solution to the alkaline tartrate solution and dilute the solution to 4 litres. Mix and leave for 1 to 3 weeks and decant from the precipitate of cuprous oxide.) Run a reagent blank at the same time by substituting 1 ml. of 3 per cent. perchloric acid for the blood filtrate. Immerse the tubes in boiling water, heat for 8 min., and then cool for 2 min. in water at room temperature. Add 2 ml. of phosphomolybdic acid reagent (prepared by dissolving 160 g. of reagent-grade phosphomolybdic acid in 2 *N* sulphuric acid and diluting to 2 litres with 2 *N* sulphuric acid; leave for 10 days at room temperature and use the supernatant liquid) and allow the reaction to proceed for 2 min. Dilute the solutions to 25 ml. with *N* sulphuric acid. Maximum colour develops within 5 min. Evaluate the colour in a photoelectric colorimeter using a filter that transmits maximally at 660 mμ after setting the reagent blank to read 100 per cent. transmission. With this method, standards in the range 10 to 80 μg. of glucose fall on a straight line with a deviation of ± 5 mg. per 100 ml. The precision of the procedure under routine conditions was ± 9·4 and ± 12·6 mg. per 100 ml. in the ranges 0 to 150 and 151 to 480 mg. per 100 ml., respectively. F. A. R.

Estimation of Glycogen in Whole Blood and White Blood Cells. R. Wagner (*Arch. Biochem.*, 1946, **11**, 249–258)—The estimation of glycogen in blood requires certain modifications of the usual technique, and special precautions have to be taken.

Method—As an anti-coagulant use 2 mg. of dry potassium oxalate per ml. of blood or, with larger amounts of blood as in the study of isolated white blood cells, 0·2 ml. of a mixture of 3 per cent. ammonium oxalate and 2 per cent. potassium oxalate solutions per 5 ml. of blood; allow this to dry on the bottom of the container in which the blood is collected. Haemolyse 1 ml. of whole blood by adding 2 ml. of water, add 3 ml. of 60 per cent. potassium hydroxide solution and heat for about 30 min. in boiling water. Cool to room temperature, add 6 ml. of water, and precipitate the glycogen with 18 ml. of 95 per cent. ethanol. Allow the precipitate to settle overnight and then centrifuge for 25 min. at 2000 r.p.m. Wash with 60 per cent. alcohol until the supernatant liquid is colourless, and hydrolyse the precipitate with 4 ml. of 2·2 per cent. hydrochloric acid for 3 hours. Neutralise with 2 *N* sodium hydroxide, using one drop of phenol red as indicator and dilute the hydrolysate to 10 ml. Centrifuge and estimate the sugar in a 9-ml. portion by Somogyi's method. Repeat the procedure with another 1-ml. sample of the whole blood, but ferment the hydrolysate for one hour with 0·5 ml. of a 10 per cent. aqueous suspension of baker's yeast prior to determining the sugar. In the analysis of isolated blood cells, transfer the total hydrolysate to a 10-ml. flask, and use 5 ml. for the direct determination and 5 ml. for the determination after fermentation. The difference between the two results is a measure of the glycogen content of whole blood or of the isolated blood cells. The fermentation is best carried out at pH 4 to 6, a convenient method being to neutralise the hydrolysate with 2 N sodium hydroxide until the phenol red turns red and then to add a 1 per cent. solution of potassium dihydrogen phosphate until a yellow-pink tinge is formed (pH 4·9).

F. A. R.

Organic

Qualitative Elementary Analysis [of Organic Substances] by Hydrogenation. A. Slooff (*Chem. Weekblad*, 1946, **42**, 34)—*Procedure*—Heat the organic compound (20 mg.) in a transparent silica tube, and carry the evolved gases by means of a current of hydrogen over a platinum spiral heated to 800° C. Pass the gases over a boat containing sodium hydroxide at 400 to 450° C., and finally through a trap containing a drop of 0·1 N hydrochloric acid coloured with methyl orange. *Carbon* is shown by a black residue from the ignition, and often by a black sublimate. The odour of naphthalene or diphenyl in the issuing gases indicates an aromatic compound. *Oxygen*—Water condensed in the connecting tube shows the presence of *oxygen*. In order to exclude false indications due to the presence of traces of moisture, the heating of the organic compound is temporarily interrupted, when, if oxygen is present, there is a rapid accumulation of small drops of water. *Nitrogen*—Ammonia is formed and caught in the trap, the contents of which may be titrated. If more than 0·1 ml. of N acid is consumed, nitrogen is present. *Sulphur*—Hydrogen sulphide is partially absorbed by the sodium hydroxde, and partially evolved in the issuing gases, where it may be detected by lead acetate paper. *Halogens*—These are held by the sodium hydroxide, and may be detected by means of silver nitrate. *Phosphorus*—Phosphorus is partially converted to phosphoretted hydrogen, and partially to phosphorus pentoxide. The former is detected in the issuing gases by moist silver nitrate paper (a positive reaction is also given by sulphur); the latter, in the sodium hydroxide. *Volatile metals*—Arsenic, antimony, cadmium, mercury, and zinc form mirrors before the platinum spiral, or behind the sodium hydroxide. Arsenic and antimony also form hydrides, which may be detected by their odours, whilst the mirrors may be dissolved in *aqua regia* and tested in the usual way. *Non-volatile metals*—These remain in the ash, which is extracted with warm water and tested for alkalies and alkaline-earths. The residue is dissolved in *aqua regia* and tested for other metals. The method may be used for liquids, and, if they are adsorbed on charcoal before analysis, for gases. Certain elements may sometimes remain in the ash in the form of salts, and the ash should therefore be tested for halides, phosphate, sulphate, and nitrate.

G. M.

Kjeldahl Determination of Nitrogen. Elimination of the Distillation. K. Marcali and W. Rieman 3rd (*Ind. Eng. Chem., Anal Ed.*, 1946, **18**, 709–710)—The determination of ammonia without distillation by the use of standard sodium hypobromite solution is inconvenient since the hypobromite solution is unstable at room temperature and must be kept between 0° and 5° C. Since ammonia reacts readily with formaldehyde to form hexamethylenetetramine, ammonium salts may be titrated with sodium hydroxide solution in the presence of formaldehyde with phenolphthalein as indicator. The method consists of oxidising the organic matter in the sample by concentrated sulphuric acid, potassium sulphate, and a mercury catalyst; neutralisation of the excess acid with alkali in the presence of sodium bromide to prevent precipitation of mercury compounds; and titration of the ammonium salt with standard alkali to the phenolphthalein end-point in the presence of formaldehyde.

Method. Reagents—0·1 N sodium hydroxide standardised against potassium biphthalate and stored in alkali-resistant glass. Neutral formaldehyde solution, approximately 18 per cent. prepared by diluting the reagent grade solution (36 to 38 per cent.) with water, adding 2 drops of 1 per cent. phenolphthalein solution, and neutralising with 0·1 N sodium hydroxide just before use.

Procedure—Take a sample containing about 4 mg.-equivalents of nitrogen, and treat it with 10 g. of anhydrous potassium sulphate, 0·6 to 0·7 g. of mercuric oxide, and 15 ml. of concentrated sulphuric acid. Heat cautiously, as usual, until 20 min. after the solution clears. Cool, and dilute with 50 ml. of water. Add 10 ml. of 60 per cent. sodium bromide solution, 2 drops of 0·1 per cent. methyl red, and neutralise with 10 N sodium hydroxide. Boil gently for 3 min. to expel carbon dioxide, cool, and add 10 N alkali until the solution is just yellow. Add N sulphuric acid until the pink colour is just restored, and titrate with standard 0·1 N sodium hydroxide; read the burette. Add 30 ml. of the formaldehyde solution, disregard any slight pink colour of the solution, and continue the titration until the solution is yellow. Add 8 drops of phenolphthalein solution and complete the titration to the first distinct pink colour. The alkali used between the methyl red and phenolphthalein end-points is equivalent to the nitrogen present. If more than 17 ml. of concentrated sulphuric acid are used, enough silica is introduced as an impurity in the sodium hydroxide during the neutralisation to buffer the phenolphthalein end-point and to prevent a sharp colour change. Determine and apply a blank correction, usually 0·20 to 0·30 ml.

Results—On eleven carefully purified organic substances a maximum mean deviation of 1 in 220

is recorded, from 2 to 6 determinations being made on each substance. Standard procedures give similar accuracy on the three compounds for which results are given by each method. Agreement between the methods is good in samples with both low and high nitrogen contents. Calcium, barium, copper, and iron interfere by forming precipitates that obscure the end-point; phosphorus interferes since it is converted from primary to secondary phosphate between the end-points, so the method is inapplicable to fertilisers and many biological samples. Use of the sulphuric acid-salicylic acid modification before digestion renders the method applicable to samples containing nitrate-nitrogen.
M. E. D.

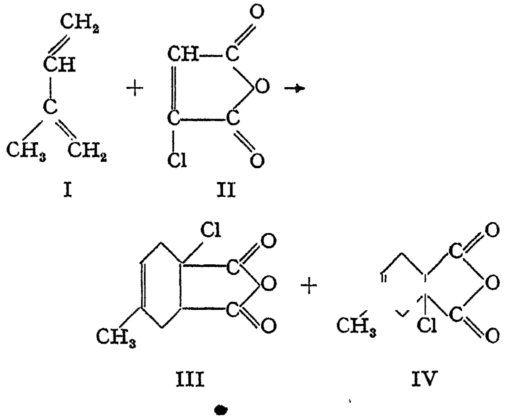
Estimation of Pentose in the Presence of Large Quantities of Glucose. A. H. Brown (*Arch. Biochem.*, 1946, **11**, 269–278)—Hexoses interfere with the orcinol reaction for the colorimetric determination of pentose. The procedure now described eliminates the interference due to as much as a 15-fold excess of hexose. The method employs light of different wavelengths in a conventional dichromatic assay.

Method—Into a series of tubes put 0·5, 1·0, and 1·5 ml. of a standard solution containing 0·1 μM of xylose or other pentose per ml., and the same volumes of a solution containing 3 μM of glucose per ml.; include with the series a distilled water blank. Make up the volume in each tube to 1·5 ml. with water. Put into other tubes the unknown solutions diluted so that the pentose concentration of each is in the range 0·03 to 0·07 μM per ml. Biological extracts should first be clarified by means of the barium - zinc method of Somogyi (*J. Biol. Chem.*, 1945, **160**, 69), rather than by the more usual lead oxalate method, which should be avoided. Dilute the unknowns to 1·5 ml., and to both standards and unknowns add 4·5 ml. of orcinol reagent. (This is prepared by dissolving 4 g. of orcinol in 100 ml. of 1·5 per cent. $FeCl_3.6H_2O$ solution and diluting 1 vol. to 20 vols. with 30 per cent. hydrochloric acid.) Immerse the tubes in boiling water for 20 min., cool, and evaluate the colour with a red filter, *e.g.*, Klett No. 66, or Corning No. 2403 alone or with Corning No. 3962, to eliminate infrared. Repeat the colour measurement with a green filter, *e.g.*, Klett No. 54, or a combination of the three Corning filters No. 5120, 4303, and 3484. It is advisable to measure the colours of any one tube with the two filters within 30 min. The results can be calculated from the simultaneous equation resulting from the two estimations, but the method is time-consuming, and it is simpler to adopt a graphical method of calculation as follows: From the series of standard hexose samples prepare for both sets of filters calibration curves relating colorimeter readings to glucose concentrations. From the readings obtained with the unknown, determine its apparent glucose content for each wavelength from this calibration curve. If pentose is present, the apparent glucose content will be different in the two instances, and the difference can be converted graphically into true pentose content from the curve obtained by plotting the colorimeter readings of the pentose standards against the difference in the apparent glucose content. The error of the method is less than ± 5 per cent. for a single estimation within the range 0·03 to 0·20 μM of pentose in 1·5 ml. of sample when not more than 1·0 μM of glucose is present. The method is applicable to the estimation of pentose in fructose-pentose mixtures.
F. A. R.

Colorimetric Estimation of Glycogen. D. L. Morris (*J. Biol. Chem.*, 1946, **166**, 199–203)—The red-brown colour formed by glycogen with iodine varies in intensity not only with the concentration of the glycogen, but also with the temperature, the iodine concentration, and the source of the glycogen. Extraordinary precautions must be used when glycogen is estimated by measurement of this colour.

Glycogen from animal and vegetable sources appeared to produce the same colour when ammonium sulphate and iodine were added, and it was impossible to distinguish between them in this way.
F. A. R.

Determination of Conjugated Diolefines with Chloromaleic Anhydride. S. T. Putnam, M. L. Moss, and R. T. Hall (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 628–630)—Chloromaleic anhydride reacts with conjugated diolefines in the same manner as maleic anhydride to form a Diels-Alder adduct. Thus, isoprene (I) reacts with chloromaleic anhydride (II) to give 1-chloro-4-methyl-4-cyclohexene-1:2-dicarboxylic anhydride (III) and 2-chloro-4-methyl-4-cyclohexene-1:2-dicarboxylic anhydride (IV).



Owing to the difference in reactivity between the highly active tertiary chlorine of the adduct and the unreactive vinyl chlorine of the reagent, a quantitative determination of the adduct in presence of excess of the reagent can be made by heating under reflux with aqueous silver nitrate, which removes the chlorine completely from the adduct, leaving the chlorine of the reagent untouched.

Preliminary investigation showed that purification of commercial chloromaleic anhydride is necessary, chloromaleic acid, formed by hydrolysis, being a very troublesome impurity, probably catalysing the dimerisation and polymerisation of the conjugated dienes, and thus rendering them unavailable for adduct formation. Even with extensive purification it is difficult to obtain a reagent giving theoretical results, and it is advisable to use a purified reagent giving values between 95 and 100 per cent. of the theoretical, and to apply an empirical correction factor determined by analysing a sample of known diene content. Low and variable results are also obtained in presence of peroxides, which catalyse the co-polymerisation of conjugated dienes with chloromaleic anhydride, and consequently a small amount of inhibitor, *e.g.*, *p*.-tert.-butylcatechol, is added as a precautionary measure in all analyses:

Procedure—To purify the commercial reagent, separate the crystals from the mother liquor by filtration, wash them with dry hexane, dissolve them in the minimum amount of dry benzene required, and add hexane to incipient turbidity at room temperature. Seed the solution and cool in an ice bath (+3° C.). Collect the recrystallised product by filtration, and distil it under reduced pressure in an atmosphere of dry nitrogen, or carbon dioxide. Collect the fraction boiling at 110° C. under 44 mm. of mercury, and seal it in small glass ampoules in dry nitrogen or carbon dioxide until required. The purified product melts at 32° to 34° C., and is completely soluble in dry benzene (chloromaleic acid is insoluble). If the correction factor, determined by analysis of a known sample, exceeds 1·05, further purification is recommended.

Introduce volatile samples into a small, tared glass bulb having a long, finely drawn-out neck by inverting the orifice in the liquid and cooling the bulb with dry ice until 0·1 to 0·2 g. of sample has been drawn into the bulb. Seal the bulb quickly in a small flame, holding a piece of dry ice just below the tip of the stem to prevent carbonisation of the sample. Reweigh the bulb at room temperature and place it in a pressure bottle with 1 ± 0·1 g. of chloromaleic anhydride. Add 1 drop of a 10 per cent. solution of *p-tert*-butylcatechol in nitrobenzene, cap the pressure bottle by means of a household bottle capper, and break the bulb by striking the bottle sharply against a suitable surface. Heat for 2 hr. at 55° C., cool, open the bottle, and transfer the contents into a 250-ml. Erlenmeyer flask through a funnel with the aid of 10 ml. of acetone and 50 to 75 ml. of water. Rinse the cap with 2 ml. of acetone. Add 20 ml. of 0·2 *N* silver nitrate, and boil the mixture under reflux for 1 hr. After cooling, filter, wash the precipitate thoroughly with water, add 5 ml. of diluted nitric acid (1 + 1), and 1 ml. of ferric alum solution to the filtrate, and titrate the excess of silver nitrate with 0·1 *N* potassium thiocyanate. From the amount of silver nitrate converted into silver chloride, calculate the amount of diene in the sample, applying the necessary correction factor. If the molecular weight of the substance is not known, an approximate molecular weight may be assumed, or the result may be expressed as diene numbers.

Non-volatile samples may be weighed directly into the pressure bottle from a weighing burette. Otherwise the procedure is the same.

Heating at 55° C. for 2 hr. is sufficient for samples containing over 25 per cent. of isoprene, or for *cyclo*pentadiene. Samples containing smaller amounts of isoprene should be heated at higher temperatures and/or for longer periods, *e.g.*, samples containing from 5 to 10 per cent. required 4 hr. at 75° C. Butadiene reacts more slowly than isoprene and samples must be heated at 55° C. for 12 hr. to obtain satisfactory results. Isoprene - butadiene mixtures containing from 5 to 10 per cent. of butadiene require 6 to 8 hr. at 55° C., and mixtures containing 20 to 25 per cent., 8 to 12 hr. The method should be applicable to samples containing less than 5 per cent. of diene, but the optimum conditions have not been established experimentally.

Styrene interferes in the determination owing to the formation of a co-polymer with chloromaleic anhydride, even in absence of peroxides. The co-polymer contains tertiary chlorine, which is determined with the chlorine of the adduct. The method has been used most extensively for the determination of isoprene, but has been applied successfully to the determination of butadiene, *cyclo*pentadiene, and other conjugated dienes. Preliminary experiments show that *trans*-piperylene and 2-methyl-1:3-pentadiene can be determined, but not 4-methyl-1:3-pentadiene, because this compound forms no Diels-Alder adduct with the reagent. It is probable that the method can be applied to other conjugated dienes by varying the conditions of adduct formation to obtain a quantitative reaction. A. O. J.

Analysis of Furfural-Water Solutions. J. Griswald, M. E. Klecka, and R. V. O. West, jun. (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 696–698)—Water in furfural is determined by observation of the cloud-point of equal volumes of furfural and a reagent consisting of a mixture of 1-hexanol and cottonseed oil. Cloud-points with cottonseed oil alone occur at undesirably high temperatures and are lowered by the presence of the hexanol; by adjusting the ratio of oil to hexanol the cloud-point may be brought to a convenient temperature level. By substituting dry furfural for 1-hexanol in the reagent the method should be adaptable to the determination of water in any other liquid that does not react with furfural.

Hughes and Acree (*Ibid.*, 1934, **6**, 123; ANALYST, 1934, **59**, 430) reported that furfural reacts with a molecular proportion of bromine rapidly and with a second molecular proportion slowly, and they developed a satisfactory bromometric method for determining furfural depending upon this principle. In applying this method to the determination of furfural in aqueous solution, it was found that the first reaction was far too slow to be satisfactory, but that in presence of hydrochloric acid and mercuric chloride it occurred sufficiently rapidly. The end-point is conveniently determined electrometrically as the e.m.f. of a calomel electrode. The e.m.f. rises sharply from about 250 to about 800 millivolts and persists at this value for a limited time when the end-point is reached. A certain excess of bromine is consumed over the stoicheiometric equivalent, but, with proper precautions and attention to details, the end-point is reproducible. Graphs of the stoicheiometric bromine equivalent plotted against the bromine consumption indicated by the potentiometric end-point are straight lines for any given concentration of bromine water, and so the reagent may be standardised by titration against pure furfural in terms of its "potentiometric normality."

Determination of water in furfural—The 1-hexanol is dried by removal of the water by distillation. Furfural for standardising is dried and redistilled under reduced pressure, the first quarter of the distillate being rejected. The cloud-point apparatus is a test tube (1 in. × 4 in.) mounted in a 250-ml. beaker of water warmed by a Bunsen burner. The test tube contains an A.S.T.M. titre test thermometer mounted in a cork and extending to about 1·25 cm. from the bottom, and a stirrer forming a loop round the thermometer. The A.S.T.M. aniline point apparatus may be used with advantage.

Procedure—To prepare the cloud-point reagent mix 0·4 part of dry 1-hexanol with 1 part of cottonseed oil (or commercial Wesson oil) in a glass-stoppered flask. Charge the apparatus with 10 ml. of the furfural sample and 10 ml. of the reagent, warm the stirred mixture until it is perfectly clear and transparent, then cool slowly with stirring until the cloud-point is observed. A haze appears as the cloud-point is approached and the liquid suddenly becomes opaque at about 0·75° C. lower. The "opaque point" is reproducible to within ± 0·1° C. and is taken as the cloud-point. Calibrate the reagent against samples of furfural containing known amounts of water. Samples containing more than 3 per cent. of water should be mixed with 1, 2, or 3 parts of dry furfural for testing. With samples containing less than 1 per cent. of water, a slight improvement in accuracy and convenience is obtained by using a reagent containing 0·3 part of 1-hexanol and 1 part of cottonseed oil.

Bromometric titration of furfural in water—The titration cell consists of a 250-ml. beaker marked at 100-ml. and equipped with a stirrer, a thermometer, a normal calomel electrode, and a reference electrode of a spiral of platinum wire sealed in a glass tube. The cell is mounted in a water-bath (*e.g.*, a 1-litre beaker) since the temperature must be kept constant to ± 0·1° C. during the titration. The e.m.f. is observed by means of a sensitive potentiometer, an electronic voltmeter or a *p*H meter of the proper range (0 to 1 volt). The bromine reagent is made approximately 0·005 *N* in bromine, 0·01 *N* in potassium bromide, and with 10 ml. of conc. hydrochloric acid per 100 ml. It should be made daily and standardised against furfural. A saturated solution of mercuric chloride in water is run through the electrode into the cell for each titration.

Procedure—Calibrate the bromine reagent against 5-mg. samples of furfural using the same titration procedure as for unknown samples. At any constant temperature the volume of reagent plotted against amounts of furfural gives a straight line with an intercept of −0·4 ml. After subtraction of 0·4 ml. from the burette reading at the end-point calculate the potentiometric normality of the reagent. With unknown samples make two titrations, the first to determine the approximate furfural content and the second with an amount of sample containing about 5 mg. of furfural. For the second titration, charge the beaker with the sample, dilute to 100 ml., add 3 ml. of saturated mercuric chloride solution, and start the stirrer. When the temperature is constant add bromine reagent equivalent to about 95 per cent. of the total amount required as fast as the burette will drain, or within 60 sec. This will cause a temporary false end-point potential. As soon as the e.m.f. has fallen below 0·75 volt, add bromine reagent in increments of about 1 per cent. of the expected total until the e.m.f. remains above 750 millivolts for 90 sec. and record this as the end-point. The bromine required depends upon the rate of bromine addition and to a great extent upon temperature. A curve is provided whereby correction may be made for differences in temperature between standard and sample. The necessity for this may be avoided by provision of a thermostat to maintain a constant temperature in the water bath.

With care and attention to detail titrations are reproducible to ± 0·5 per cent. of the total furfural in a 5-mg. sample. Samples containing 4 or 6 mg. of furfural titrated with reagent standardised against 5 mg. of furfural yield results with an accuracy of ± 1·0 per cent. The method will obviously give high results with samples containing impurities that react with bromine. A. O. J.

Inorganic

Determination of Sodium and Potassium in Silicates. S. Kallmann (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 678–680)—When a 20 per cent. solution of hydrogen chloride in dry *n*-butyl alcohol (Willard and Smith reagent) is added to a butyl alcohol solution of the perchlorates of sodium and potassium, the alkali metals are precipitated quantitatively as a mixture of sodium chloride, potassium chloride, and potassium perchlorate. The precipitation may be carried out in the presence of most of the elements encountered in silicate analysis and is proposed as the basis of a new method of determining sodium and potassium.

Method. Reagent—Pass dry hydrogen chloride into cooled, anhydrous *n*-butyl alcohol until the solution is practically saturated. The specific gravity of a 20 per cent. solution is about 0·905.

Procedure. Decomposition of the sample—Weigh 0·5 g. or more of finely ground sample into a platinum dish, moisten, add 20 ml. of 47 per cent. hydrofluoric acid and 15 ml. of 72 per cent. perchloric acid, stir, and heat until copious fumes of perchloric acid are evolved. Cool somewhat, rinse down the sides of the dish, and heat again until about half the perchloric acid has been expelled. Cool, and dissolve the salts by adding 20 ml. of hot water and heating. An insoluble residue at this stage may be unattacked sample, or may indicate the presence of large amounts of titanium, or of barium sulphate. Filter and wash the residue with hot water. If it is suspected that the residue contains undecomposed sample ignite it in the dish, treat again with hydrofluoric and perchloric acids, evaporate, dilute, and filter into the main solution. Evaporate the filtrate and heat gently until most of the perchloric acid is expelled.

Some silicates, especially those of high alumina content, or containing much titanium, barium, or lead, are not readily decomposed by hydrofluoric acid. For these, conduct a Lawrence Smith decomposition, add 10 ml. of perchloric acid to the water extract, and evaporate almost to dryness.

Precipitation of sodium and potassium—Add 25 ml. of *n*-butyl alcohol to the perchlorates and boil. (A precipitate at this stage consists of potassium perchlorate and its amount may give a useful guide to the potassium content of the sample.) Add 3 ml. of Willard and Smith reagent dropwise to the boiling solution, and then a further 8 ml. Simmer for 3 minutes, cool, and decant the liquid through a dry Gooch crucible. Rinse the bulk of the precipitate into the crucible and wash 8 to 10 times with 1- to 2-ml. portions of wash solution (40 ml. of reagent diluted with 100 ml. of butyl alcohol.

Separation of sodium and potassium—Dry the beaker and crucible and dissolve the precipitate in the minimum amount of hot water. Add 5 ml. of perchloric acid and evaporate almost to dryness. Cool, add 15 to 20 ml. of a mixture of equal volumes of butyl alcohol and ethyl acetate, heat nearly to boiling for 2 to 3 min., and cool. Decant the liquid into a dry Gooch crucible, catching the filtrate in a small beaker. Wash the residue of potassium perchlorate 3 times by decantation with the mixed solvent. Dissolve the residue in the crucible in the minimum amount of hot water, catching the solution in the precipitation beaker. Add 1 ml. of perchloric acid and evaporate to dryness, expelling any acid condensed on the beaker walls by brushing with a flame. Cool, add 3 to 5 ml. of water, and again evaporate to dryness. Add 10 to 15 ml. of the mixed solvent, heat nearly to boiling for 2 to 3 min., and cool. Filter through the Gooch crucible which has meanwhile been dried, ignited, and weighed. Wash 8 to 10 times with 1-ml. portions of mixed solvent. Add the filtrate and washings to the filtrate from the previous precipitation. Dry the Gooch crucible and precipitation beaker and brush any remaining particles of potassium perchlorate into the Gooch crucible. Dry the crucible for 1 hour at 110° C. and finally, covered by a watch-glass, for 15 min. at 350° C. Cool, and weigh as potassium perchlorate.

Determination of sodium—An estimate of the amount of sodium present may be made by observing the effect of the addition of the Willard and Smith reagent in separating the sodium and potassium from the non-alkali constituents of the sample. If the amount is small, add one-third of its volume of water to the combined filtrate from the potassium precipitation and evaporate to dryness. Add 10 ml. of water, 1 ml. of nitric acid, and 1 ml. of sulphuric acid and evaporate to fuming. Add more nitric acid, if necessary, to destroy organic matter and then heat until all the sulphuric acid is removed. Add water, filter if necessary, evaporate to 1 ml. and determine the sodium by a standard triple acetate method.

If the amount of sodium present is large, evaporate the filtrate to dryness as above. Add 10 ml. of water and 3 ml. of perchloric acid, cover the beaker, and heat until fumes of perchloric acid are copiously evolved. When all organic matter is destroyed, evaporate almost to dryness. Add 15 to 20 ml. of butyl alcohol and boil. Add 7 to 9 ml. of Willard and Smith reagent, the first 2 ml. dropwise, simmer for 1 min. and filter through a dry Gooch crucible and wash 5 times with 1- to 2-ml. portions of wash solution. Dry the crucible at 110° C. for 0·5 hr., and then heat for 5 min. at 650° C. Cool and weigh. Dissolve the precipitate in hot water, wash, dry, and reweigh the crucible. The loss of weight represents the sodium chloride.

The solution may be tested for barium or lead and purified either by adding ammonium carbonate, filtering and recovering sodium as the chloride or by adding sulphuric acid, filtering and recovering the sodium as the sulphate.

For a rapid routine method the mixed alkali precipitate may be collected on a Gooch crucible and converted to chlorides by heating at 650° C. The residue is then dissolved and either potassium or sodium determined by any convenient method, the other element being found by difference. L. A. D.

Determination of Calcium in Magnesite and Fused Magnesia. W. M. Hazel and W. K. Eglof (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 759–760)—In the method proposed for the determination of calcium oxide in materials containing much magnesium oxide, mannitol is used to prevent precipitation of the calcium during precipitation of the magnesium as hydroxide. The calcium is finally precipitated as oxalate and weighed as lime.

Procedure—In a 100-ml. porcelain dish dissolve a weighed portion of about 2 g. of the magnesite or fused magnesia in 35 ml. of diluted hydrochloric acid (1+1). If the material is not completely soluble in the acid, mix a 2-g. sample with 3 to 5 g. of sodium carbonate in a platinum crucible, and heat at 900° to 1000° C. for 30 min. Dissolve the cold melt by adding to it 50 to 60 ml. of diluted hydrochloric acid (1+1), in the platinum crucible. After dissolution of the sample, evaporate the solution to dryness on a steam-bath, and bake the residue for one hour at 120° C. Digest the residue with 5 ml. of concentrated hydrochloric acid and 50 to 75 ml. of hot water. Filter off the silica and wash the precipitate with hot, diluted hydrochloric acid (1+4) and finally with hot water. Transfer the cold filtrate to a 500-ml. graduated flask, add 15 to 20 g. of mannitol, 6 drops of phenolphthalein solution, and then add dropwise, carbonate-free, 9 *N* sodium hydroxide until the solution is coloured pink. Add dropwise, while shaking the flask, a further 5 ml. of the sodium hydroxide solution. Cool to room temperature, dilute to 500 ml., allow to stand overnight, pour the liquid through a loose, dry filter paper, and transfer a 200-ml. portion of the filtrate to a 400-ml. beaker. Add 5 drops of methyl red and acidify with hydrochloric acid, adding 5 ml. in excess. If much magnesium is present in this solution, add 5 g. of ammonium chloride. To the boiling solution add 0·5 g. of ammonium oxalate, and aqueous ammonia until the solution is neutral. Digest on a steam-bath until the calcium oxalate is well formed, remove, add dropwise 2 ml. of aqueous ammonia, and allow to stand for 4 hr. Filter through a No. 40 Whatman paper, and wash the precipitate with cold, 1 per cent. ammonium oxalate solution. Dissolve the precipitate by washing the paper with 50 ml. of hot, diluted hydrochloric acid (1+2). Reprecipitate the calcium oxalate, collect the precipitate, and wash it, following the method used for the first precipitation. Ignite the oxalate in platinum at 1100° to 1200° C. and weigh as calcium oxide (*cf.* Scott, W., "*Standard Methods of Chemical Analysis*," New York, 1939).

Solutions equivalent to those of samples of magnesia containing known weights of from 0·5 to 5 per cent. of calcium oxide were analysed by the method. For weights of lime from 4 to 40 mg., the errors in the analyses were not greater than ± 0·2 mg. Several samples of magnesite, including Bureau of Standards Samples, were analysed and the highest probable error found was ± 0·6 mg. for 26·8 mg. of lime, the results obtained showing the method to be accurate and trustworthy. The proposed method gives results slightly higher than those obtained by the usual method in which calcium sulphate is precipitated in the presence of alcohol. Small quantities of aluminium do not interfere. Spectrographic analyses of a number of samples of lime obtained by this method showed them to be of analytical purity and free from co-precipitated constituents of the materials investigated. B. A.

Improved Analysis of Fuming and Concentrated Sulphuric Acid by Water Titration. J. C. D. Brand (*J. Chem. Soc.*, 1946, 585–588)—The alkalimetric determination of "free" sulphur trioxide in oleum incurs relatively large errors since most of the alkali is required by the sulphuric acid and only a small fraction is consumed by the sulphur trioxide. Titration with water until the cooled liquid no longer fumes in air can be used to determine the sulphur trioxide directly and more accurately. Modifications of Parker's procedure (*J. Soc. Chem. Ind.*, 1917, **36**, 692) now suggested are (1) a smaller titration drop and (2) a temperature below 10° C. at the end-point of the titration (anhydrous sulphuric acid fumes perceptibly at 13° C.)

Procedure—Using water in a micro-burette graduated to 0·02 ml. and with a jet drawn out to deliver 0·01-ml. drops, titrate 25 to 150 g. of oleum, according to its strength, in a glass-stoppered conical flask. Add the water in such an amount at a time that there is no loss of fume, and absorb the fume within the flask by vigorous shaking after each addition. Cool the flask in ice so that the temperature at the end-point of a titration is less than 10° C. Near the end-point fuming decreases, and is tested for by blowing a puff of air through a tube held a few millimeters above the surface of the liquid and inspecting the surface in bright daylight, the flask being held against a dark background. Immediately before the end-point a wisp of fume develops and lies on the centre of the oleum surface. If v ml. of water are consumed by 100 g. of oleum, then the strength of the oleum is $(100+v)$ per cent. of sulphuric acid, or the percentage of "free" sulphur trioxide is $v/0{\cdot}225$.

Agreement between this method and that involving titration with alkali is satisfactory, but the validity of the new method has been established by determinations of the freezing points of the acids obtained by adding water to an oleum containing 1·00 per cent. of "free" sulphur trioxide, and by showing that the maximum point obtained at 10·5° C. on the freezing point - water content curve corresponds to the point at which fuming ceases abruptly, the freezing point of anhydrous sulphuric acid being lowered by the addition of either water or sulphur trioxide.

Determination of the strength of concentrated sulphuric acid—Attempts to analyse concentrated sulphuric acid by direct titration with oleum to the first appearance of a fume were unsatisfactory, but the back-titration method can be used.

Procedure—Mix a weighed quantity of oleum of known strength with a weighed amount of concentrated sulphuric acid, and determine the remaining free sulphur trioxide by titration with water. The difference between the titration of the oleum and of the mixture of oleum and concentrated acid is equal to the volume of water contained in the aqueous acid, from which its strength can be calculated. The method gives the water content of concentrated sulphuric acid to within 0·2 per cent. of sulphuric acid, an accuracy several times greater than that of the volumetric alkalimetric method.

Preparation of standard aqueous sulphuric acid—Suitable dilution of a weighed quantity of a weak oleum containing about 1 per cent. of "free" sulphur trioxide, which can be done without significant loss of fume, gives approximately *N* solutions of a concentration known accurately to 1 part in 10,000, since this is the accuracy with which an oleum can be analysed by the new water-titration method.

M. E. D.

Nitration in Sulphuric Acid. Part III. Influence of Nitric Acid and other Substances on the Oleum-Water Titration. J. C. D. Brand *J. Chem. Soc.*, 1946, 880–884)—Water titrations have been carried out on oleum solutions of various solutes and the resulting variations in titre studied. Potassium sulphate, potassium bisulphate, barium sulphate, potassium dihydrogen phosphate, nitric acid, potassium nitrate, sodium nitrite, and barium nitrate all reduce the titre by an amount directly proportional to the solute concentration, and independent of the initial free sulphur trioxide concentration; 2:4:6-trinitrotoluene and 1:3:5-trinitrobenzene have no significant effect. It is found that the reduction in moles of water required per mole of solute added is 0 for the trinitro compounds, 0·5 for potassium bisulphate, 1·0 for potassium and barium sulphates and potassium dihydrogen phosphate, 1·5 for nitric acid, 2 for potassium nitrate and sodium nitrite, and 4 for barium nitrate. The diminution in titre is a measure of the combination between the solute and the sulphur trioxide of the oleum, and is used in interpreting the reactions that may occur when nitrate, nitrite, and hydrogen sulphate ions are added to oleum. M. E. D.

Rare-Earth Metal Oxides. Part II. New Study of Oxide Precipitation by Nitrate Fusion. J. K. Marsh (*J. Chem. Soc.*, 1946, 17–20)—If anhydrous cerous nitrate is added to a cerium-free melt of rare-earth nitrates and alkali nitrates, cerium dioxide is precipitated in the melt and carries down with it a large proportion of the praseodymium present. By precipitation of praseodymium in this manner, it is possible to obtain 96 per cent. pure lanthanum compound from a mixture of lanthanum and praseodymium nitrates.

Praseodymium and neodymium nitrates were estimated with the Hilger "Spekker" Absorptiometer, 1-cm. cells being used. For neodymium, a green light filter (Ilford 604) was used, and it was possible to estimate 0 to 100 g. per l. with an accuracy of 1 g. per l. In the estimation of praseodymium, yellow and orange filters (Ilford 606 and 607) were used together, and a water setting of 0·5 was employed. Amounts of 0 to 100 g. per l. could be estimated with an accuracy of 2 g. per l. For the estimation of praseodymium in the presence of neodymium, filters of a solution of neodymium nitrate, of concentration equivalent to 400 g. per l. of neodymium oxide, were used with a violet filter (Ilford 601). Praseodymium has its main absorption band in the middle of the spectral range, 4540 to 4340 A, covered by the band of light transmitted by this combination of filters. The absorption effect of neodymium was about 3 per cent. of that of praseodymium and a correction for this was applied. From 0 to 30 g. of praseodymium per l. could be estimated to within ± 1 g. per litre.

B. A.

Rare-Earth Metal Oxides. Part III. Their Precipitation from Potassium Hydroxide Melts. J. K. Marsh (*J. Chem. Soc.*, 1946, 20–23)—Rare-earth hydroxides can be peptised in molten potassium hydroxide containing 16 per cent. of water. On desiccation of the melt at high temperatures, and with oxidising conditions, praseodymium dioxide and terbium dioxide are the first oxides to be precipitated. If a dysprosium hydroxide containing terbium hydroxide is peptised in this type of melt and the melt desiccated, an efficient elimination of the terbium from the dysprosium is obtained. A rapid, partial separation of praseodymium from neodymium can be effected by the same method.

B. A.

Use of Ion Exchange Resins in the Study of Chrome Liquors. R. S. Adams (*J. Amer. Leather Chem. Assoc.*, 1946, **41**, 552–573)—This paper describes an investigation of the possibility of using ion exchange resins in the analysis of the solutions of chromium salts that are used in tanning. Two columns containing ion exchange resins were used, one containing a cation exchanger that removed chromium ions, and cationic complexes containing chromium, from the solutions, and the other containing an anion exchanger that gave up chloride ions in exchange for other anions, including anionic chromium complexes. Solutions of chromium chloride, chromium sulphate, and chromium oxalate, alone, and with sodium salts added, were used in the experiments, one of the chromium sulphate solutions being prepared by reduction of sodium chromate solution by glucose. The solutions were passed through the cation exchanger, the effluent was analysed for chromium, the anions present, and for total acidity; and the amount of cationic chromium present and the amount of anion bound in cationic complexes were calculated. Other portions of solutions were passed through the anion exchanger resin and analysed for chromium, the amount of chromium bound in anionic complexes being so determined.

Method—The resin is contained in a Pyrex glass tube 625 mm. long and 25 mm. in diameter, constricted at the bottom and attached to a Y-tube, both arms of which are closed by rubber tubing and screw clips. Near the top of the main tube is a side tube for overflow. The column of 55 to 60 g. of resin is supported by a piece of porcelain plate, a pad of glass wool, and a 25-mm. layer of washed gravel.. The column is filled with cation exchanger resin (Amberlite IR–100), or anion exchange resin (Amberlite IR–4).

Procedure—Add a measured volume of the chromium solution at a rate of about 5 ml. per minute for the cation exchanger, or 20 ml. per minute for the anion exchanger, the rate being so adjusted that the liquid level remains just above the top of the column of resin. When all the chromium solution has been added, maintain the level of the liquid until the desired amount of effluent has been collected, by adding distilled water. The first 50 ml. of effluent contain no chromium and may be discarded. Collect and analyse the next 200 ml. of effluent. If the anion and cation columns are run in series, collect the effluent from the first column in 25-ml. portions and pass those portions that show a chromium colour through the second column first. To regenerate the columns pass, at the rate of 5 ml. per minute until all the chromium is removed, 10 per cent. sulphuric acid solution through the cation exchanger resin, and 10 per cent. hydrochloric acid solution through the anion exchanger resin. Wash at a rate of 30 to 40 ml. per minute with distilled water until all excess of acid is removed; and finally, force 500 ml. of distilled water up the column from the side arm of the Y-tube at such a rate as to cause a 50 per cent. expansion of the resin column.

Results for the amounts of chromium ions absorbed are reproducible to within about 1 part in 35, both columns being efficient absorbers, although the anion column did not give up chloride ions quantitatively in exchange for the anions absorbed. When cationic chromium is removed from a chromium sulphate solution, the anionic chromium complexes present dissociate slowly in an attempt to re-establish equilibrium. The rate of dissociation is such as to lead to an appreciable error in the analyses of chromium sulphate solutions by this method. The method is capable of showing the approximate distribution of the chromium in a solution between the anionic and the cationic states and of revealing the approximate proportion of cation to anion in the cationic complex. B. A.

Volumetric Determination of Water in Paints and Varnishes. M. H. Swann (*Ind. Eng. Chem., Anal. Ed.*, 1946, 18, 799–800)—The water content of paints and varnishes can be determined accurately and quickly by a volumetric method that uses the Karl Fischer reagent (Fischer, *Angew. Chem.*, 1935, 48, 394).

Method—The apparatus used to determine the end-point of the titration consists of a tungsten-platinum electrode connected to a *p*H-meter. The electrode holder carries a stirrer.

Reagent—Prepare a 0·1 per cent. solution of water in pyridine and standardise this solution by titrating it into the Karl Fischer reagent to be used in the determination.

Procedure—Weigh 10 to 20 g. of the paint into a 250-ml., glass-stoppered flask. From a burette add an amount of anhydrous pyridine not less than 10 ml. for thinned samples and up to 25 ml. for unthinned samples. Swirl the mixture, add immediately 25 to 50 ml. of the Karl Fischer reagent, stopper the flask, and allow it to stand in an anhydrous glycol bath at 50° C. for 45 min.; swirl at 10-min. intervals. When the flask is cold, titrate the unreacted Karl Fischer reagent with the standard 0·1 per cent. solution of water in pyridine.

Weighed quantities of a 1 per cent. solution of water in pyridine were added to samples of paints and varnishes and the water contents of the mixtures determined by this method. The values obtained for the percentages of water recovered were correct to within ±1 part in 100. They were much more accurate than those obtained by the azeotropic distillation method. Zinc oxide was the only constituent of enamels found to interfere.

B. A.

Agricultural

Determination of 1:2-*bis*(*p*′-Chlorophenyl) Ethane in DDT Dusts and Oil Solutions. J. B. LaClair (*Ind. Eng. Chem., Anal. Ed.*, 1946, 18, 763–766)—Cristol (*J. Amer. Chem. Soc.*, 1945, 67, 1494) has suggested that the difference in reaction rates of *p,p'*-DDT and *o,p'*-DDT when subjected to dehydrochlorination might be used for determination of the *p,p'*-isomer in technical DDT. This reaction has been investigated and procedures applicable to most commercial DDT dusts and sprays have been developed. Samples (1 g.) of technical and purified DDT of known *p,p'*-DDT content were dissolved in 75 ml. of 95 per cent. alcohol and were allowed to react for 15 min. with 20 ml. of N alcoholic sodium hydroxide at 25° ± 0·1° C. Graphs of the amount of chloride ion formed (ordinate) and the percentage of *p,p'*-DDT gave straight lines corresponding to the equation (I) (chloride ion per cent. × 11) − 9·3 = *p,p'*-DDT per cent. Samples (0·5 g.) dissolved in 100 ml. of kerosene and allowed to react with 20 ml. of N alcoholic sodium hydroxide at 25° ± 0·1° C. while being stirred at 700 to 800 r.p.m. also gave linear graphs corresponding to the equation (II) (chloride ion per cent. × 23) − 16·9 = *p,p'*-DDT per cent.

Procedure for purified and technical DDT—Heat under refluxing conditions a 1-g. sample with 75 ml. of 95 per cent. alcohol until solution is effected, cool, maintain in a thermostatically controlled water-bath at 25° ± 0·1° C., add 20 ml. of N alcoholic sodium hydroxide also at the same temperature, and mix by rotating the flask at 5-min. intervals. After 15 min., quickly stop the reaction by adding 15 ml. of diluted nitric acid (1 + 3), remove the flask from the bath, add 30 ml. of 0·1 N silver nitrate, 3 ml. of 10 per cent. ferric alum solution, and 5 ml. of nitrobenzene. Shake the stoppered flask for a few seconds, rinse the stopper, and titrate the excess of silver nitrate with 0·1 N ammonium thiocyanate. From the percentage of chloride ion found calculate the percentage of *p,p'*-DDT from equation (I).

Procedure for dust mixtures not containing sulphur or organic thiocyanates—The total DDT content must first be found. Samples containing from 5 to 80 per cent. of total DDT can be ignited in a Parr peroxide bomb, or heated under refluxing conditions with metallic sodium and *iso*propyl alcohol (Umhoefer, *Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 383), and the total DDT calculated from the chloride ion formed. Samples containing 1 to 5 per cent. are most conveniently analysed by the method of Gunther (*Ibid.*, 1945, **17**, 149; ANALYST, 1945, **70**, 270), 5 to 10 g. of sample being heated under refluxing conditions with 30 ml. of N alcoholic sodium hydroxide, cooled, and acidified with nitric acid, the chloride ion being then determined as already described. The percentage of total DDT divided into 100 gives the sample weight in grams equivalent to 1 g. of total DDT. This sample weight is submitted to the procedure described for analysing technical and purified DDT, and the amount of *p,p'*-DDT in the sample is thus obtained.

Procedure for dust mixtures containing sulphur—These are first analysed for total DDT, a Parr bomb being used. With samples containing only 1 to 10 per cent. of DDT with 50 to 90 per cent. of sulphur the Gunther method is modified as follows. Heat under refluxing conditions a sample, large enough to give an accurate titration, for 10 min. with 25 to 40 ml. of acetone, cool it in an ice bath or refrigerator for 1 hr. to reduce the solubility of sulphur in acetone, remove the sulphur by suction through a small Buchner funnel, and wash it twice with cold acetone. Heat the filtrate on a steam bath with the aid of a current of air only long enough to remove the acetone. Heat under a refluxing condenser the residue of DDT with 30 ml. of N alcoholic sodium hydroxide for 15 min., cool, add a measured excess of 0·1 N silver nitrate, 50 ml. of water, and a few carborundum chips, and boil gently until most of the alcohol has been removed. Cool slightly, add 20 ml. of diluted nitric acid (1+1) and boil the mixture until all the silver sulphide has been decomposed. Determine the chloride ion by titration with 0·1 N ammonium thiocyanate as before, and calculate the total DDT content by means of the factor 3·546. To determine the *p,p'*-DDT content heat under refluxing conditions a sample weight equivalent to 1 g. of total DDT with 40 ml. of acetone for 10 min., cool in an ice bath or refrigerator, remove the sulphur as before, and remove acetone from the filtrate. Heat the residue of DDT under reflux with 75 ml. of 95 per cent. alcohol, cool, and dehydrochlorinate at 25° ± 0·1° C. for 15 min. with 20 ml. of N alcoholic sodium hydroxide. Stop the reaction by adding 15 ml. of diluted nitric acid (1+3), add 30 ml. of 0·1 N silver nitrate, 50 ml. of water and a few carborundum chips, heat to remove most of the alcohol, add 20 ml. of diluted nitric acid (1+1), and boil to decompose silver sulphide. Determine the chloride ion by titration with 0·1 N ammonium thiocyanate and calculate the amount of *p,p'*-DDT as in mixtures not containing sulphur.

Procedure for dust mixtures containing organic thiocyanates—Total DDT may be determined by ignition in a Parr bomb. With samples of low DDT content the Gunther method must be modified to prevent inclusion of cyanide and cyanate ions in the determination of chloride ions. Heat the sample under reflux with alcoholic sodium hydroxide, filter the liquid into a separating funnel and wash the filter thoroughly. Acidify the filtrate slightly with diluted nitric acid and extract twice with light petroleum to remove the dichloroethylene derivative of DDT, which would otherwise yield chloride in the subsequent boiling with nitric acid. Drain the aqueous layer into a 400- to 600-ml. beaker and combine with it two aqueous washings of the petroleum layer. Add a measured amount of 0·1 N silver nitrate, 25 ml. of concentrated nitric acid, and a few carborundum chips. Cover the beaker with a watch glass, boil and evaporate the solution nearly to dryness to decompose silver cyanide and cyanate. Cool, and wash the liquid into a 250-ml. flask and titrate with 0·1 *N* ammonium thiocyanate as before, and calculate the percentage of total DDT as for mixtures not containing sulphur. To determine the content of *p,p′*-DDT, dehydrochlorinate a sample weight equivalent to 1 g. of total DDT with 20 ml. of alcoholic sodium hydroxide at 25° ± 0·1° C., stopping the reaction after 15 min. with diluted nitric acid. Filter into a separating funnel without suction, wash the filter free from soluble chloride, and extract the combined filtrate and washings twice with light pertroleum to remove the DDT olefine. Draw the aqueous layer into a 600-ml. beaker and add to it two aqueous washings of the petroleum layer. Add 30 ml. of 0·1 *N* silver nitrate, 25 ml. of concentrated nitric acid, a few carborundum chips, boil almost to dryness, cool slightly, and repeat the evaporation with 10 ml. of nitric acid. Cool, wash the liquid into a flask with water, and titrate the excess of silver nitrate as before and calculate the percentage of *p,p′*-DDT.

Procedure for oil solutions—Heat a sample of the oil solution weighing from 5 to 20 g. with 30 ml. of *N* alcoholic sodium hydroxide for 15 min. under reflux, cool, acidify slightly with nitric acid, and transfer into a separating funnel. Drain the lower layer into a 250-ml. Erlenmeyer flask, adding to it two aqueous washings of the upper layer. Determine the chloride ion in the aqueous layer and calculate the total DDT by means of the factor 3·546. In presence of organic thiocyanates, heat the sample under reflux as described, cool, transfer to a separating funnel, acidify with nitric acid, and extract twice with light petroleum to remove the DDT olefine. Drain the lower layer into a 600-ml. beaker together with two subsequent aqueous washings of the upper layer, cover the beaker, boil, and evaporate the liquid nearly to dryness. Determine the chloride ion and calculate the total DDT by means of the factor 3·546. To determine *p,p′*-DDT in the absence of organic thiocyanates weigh an amount of sample equivalent to 0·5 g. of total DDT, calculate its volume from its density, and make it up to 100 ml. with kerosene. Maintain the liquid at 25° ± 0·1° C. and, while it is stirred at 700 to 800 r.p.m., add 20 ml. of *N* alcoholic sodium hydroxide at the same temperature and allow the reaction to proceed for 15 min. before adding diluted nitric acid (1 + 3). Stop the stirrer, rinse the liquid into a separating funnel, and determine the chloride content of the lower layer as already described. Obtain the percentage of *p,p′*-DDT by means of equation (II). For determination of *p,p′*-DDT in presence of organic thiocyanates the sample and kerosene are dehydrochlorinated as already described, 20 ml. of *N* alcoholic sodium hydroxide being added while the liquid is stirred at 700 to 800 r.p.m. The reaction is allowed to proceed for 15 min. and then stopped by addition of 15 ml. of diluted nitric acid (1 + 3). The reaction mixture is washed into a separating funnel and the separated aqueous layer is treated as in the determination of total DDT in presence of organic thiocyanates. The *p,p′*-DDT content is calculated from the chloride formed by means of equation (II).

The values for *p,p′*-DDT obtained by the method are relative to the crystallisation method (Cristol *et al.*, *J. Amer. Chem. Soc.*, 1945, **17**, 470) as standard. An accuracy of 2 per cent. of *p,p′*-DDT can be expected with dry mixtures and 4 per cent. with oil mixtures. Pyrethrins can cause an error of 5 per cent. in dust mixtures. Water soluble chlorides, when present, must be determined and allowed for; if they occur in large amounts it is advisable to extract the DDT with benzene and determine the total DDT in the benzene extract.

A. O. J.

Gas Analysis

Determination of Hydrogen Sulphide in Gases. E. Field and C. S. Oldach (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 665–667)—A sensitive quantitative test for hydrogen sulphide is furnished by the technique described, which enables the optical detection of 1·4 µg. of hydrogen sulphide in aqueous solution, the two methods of procedure being particularly suited to the identification and determination of organic sulphur compounds present as impurities in commercial gases and solvents. The sulphide-bearing gas is scrubbed with 6 per cent. sodium hydroxide solution, and the resulting sulphide is precipitated as bismuth sulphide, the concentration of the suspension being determined by measuring the transmission of monochromatic light with an ultra-violet spectrophotometer. The accuracy is ± 10 per cent. when 7 µg. of hydrogen sulphide are collected, but by increasing the amount absorbed, the accuracy can be raised to ± 3 per cent. A sensitivity to 7 µg. can be attained by precipitating cadmium sulphide in the presence of uranyl ions, and comparing the colour and opacity with a blank in a visual comparator.

Method. Reagents—Prepare a 6 per cent., oxygen-free sodium hydroxide solution, and a solution of bismuth nitrate in diluted acetic acid (1 + 5), containing about 1 g. of bismuth per litre.

Uranyl-cadmium reagent—Dissolve 44·4 g. of uranyl nitrate hexahydrate and 31·4 g. of cadmium acetate dihydrate in 24 l. of diluted acetic acid (1 + 5).

For calibration purposes prepare a solution of about 3 g. of sodium sulphide nonahydrate in 1 litre of 6 per cent. sodium hydroxide solution, and determine its concentration iodimetrically (Shaw, *Ibid.*, 1940, **12**, 668). Dilute aliquot portions with 6 per cent. sodium hydroxide solution to provide solutions of known concentration for the calibrations.

Scrubbing hydrogen sulphide from the gas stream—Oxidation of the sulphide is avoided by the absence of oxygen, but up to 1 per cent. of oxygen is removed by the conversion procedure described by Field and Oldach (*Ibid.*, 1946, **18**, 668, ANALYST, 1947, **72**, 115). Hydrogen, nitrogen, carbon monoxide, methane, ethylene, and up to 2 per cent. of carbon dioxide do not interfere. Absorption of hydrogen sulphide is complete using 6 per cent. sodium hydroxide solution while maintaining a gas rate of 0·6 litre per min. For the comparator method 100 ml. of solution in a Milligan bubbler are used, but 10 ml. are sufficient in the spectrophotometric method.

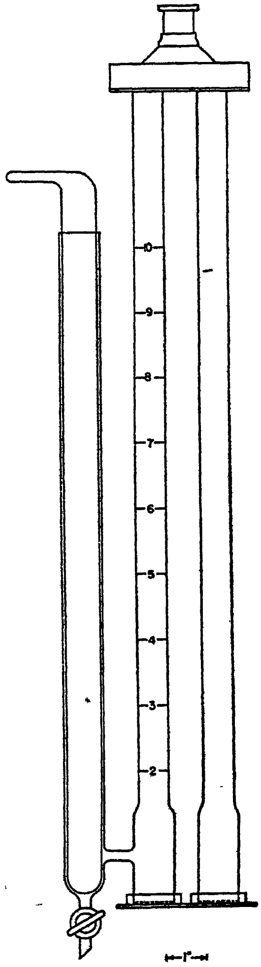
General precautions—Greased joints must be avoided. Use of a nitrogen atmosphere in transferring the solutions is also an advantage. On mixing the solutions, considerable changes occur due to reaction effects, but they become slow after about 5 min. The determination takes about 10 min. to complete, but any tendency for the precipitate to settle during that time can be reduced by adding 0·2 per cent. of clear gelatin to the bismuth reagent (J. K. Fogo, private communication). The final reagent concentrations are not critical, but the solution should be weakly acid.

Spectrophotometric method. Procedure—To the alkaline sulphide solution add an equal volume of bismuth reagent, mix thoroughly by passing oxygen-free nitrogen for 30 sec., and set aside for exactly 5 min. Transfer 10 ml. of the suspension to a spectrophotometer cuvette, the spectrophotometer being arbitrarily balanced at 100 per cent. transmission without the cuvette. Exactly 7 min. after precipitation determine the transmittance at wavelength 350 to 355 mμ with the cuvette in place, and compare with the calibration chart. The alkaline solution should contain between 6 and 40 μg. of sulphur in 10 ml.

A straight line is obtained when the sulphur concentrations of the calibration solutions are plotted against the logarithm of the percentage transmittance. The slope of the line is constant, but the intercept at zero sulphur concentration varies slightly. Thus, once the slope is determined the unit may be calibrated for zero sulphur content by mixing equal volumes of 6 per cent. alkali and the bismuth reagent.

Visual method. Apparatus—A standard comparator tube was used for the blank solution, while the second tube was of special design with a levelling tube and plunger (see Fig.). The light-source was a mercury-vapour lamp, and mounted between it and the comparator were two 2-in. square filters, resulting in the transmission of only blue and green light.

Procedure—Prepare a blank solution by mixing equal volumes of 6 per cent. sodium hydroxide solution and the uranyl-cadmium reagent, and pour it into the blank tube to the top of the scale. Take a portion of the standard solution containing from 30 to 200 μg. of sulphur, and dilute to 70 ml. with 6 per cent. alkali; mix the solutions by passing nitrogen. Add 70 ml. of the uranyl reagent and stir as before. Pour into the comparator tube and leave for 3 min.* Then adjust the plunger so that the colour and intensity match. Repeat with aliquot portions of different sizes. A graph of the logarithm of the balancing height against the sulphur content gives a straight line with a gentle curve near the origin. The curve varies slightly and should be prepared each day. The absorbing solution should contain 30 to 200 μg. of sulphur in 70 ml. to fall in the range of the comparator. Results are reproducible, but a personal factor is



involved. Figures obtained by both methods are given for each sample analysed, and show deviations ranging from −1·9 to +2·3 on values of about 22. Most of the results are within ± 1·0, the units being arbitrary.

The improvement of the accuracy in the presence of uranyl ions occurs only in the use of the comparator. The sulphide-free alkali solution transmits both the green and blue light penetrating the filters, but addition of sulphide causes absorption of the blue, the degree depending on the concentration and height of the liquid column. The use of a plunger is advantageous in that the balance point can be approached from both sides, thus increasing the accuracy over methods in which stop-cocks are used to establish liquid levels. M. E. D.

Physical Methods, Apparatus, etc.

Flame Method of Spectrochemical Analysis. W. A. Roach (*J. Soc. Chem. Ind.*, 1946, **65**, 33–39)—A rapid routine procedure, based on a method due to Ramage (*Nature*, 1929, **123**, 601), is described for the determination of certain elements in plants.

The leaves are the most useful parts for analysis; they are first cleaned, wet methods being avoided if possible owing to the risk of leaching action of the distilled water or solvents used. The cleaned leaves are slowly dried and then ground to a fine powder while still warm. A weighed amount, usually 25 mg., of plant powder is folded into Whatman No. 541 filter paper to form spills which are made to pre-determined dimensions by the use of special jigs. These jigs are designed so that there is no risk of the filter paper coming in contact with the fingers.

The spills are then charred in ammonium chloride vapour at approximately 350° C. This treatment converts the constituents of the material to the corresponding chlorides, thus increasing the sensitivity of the method. The spill, placed in a platinum trough, is pushed into an oxy-acetylene blowpipe flame at a constant speed by a wooden pusher geared to a synchronous motor, a constant length of the spill being burnt during each operation. The spectrogram is prepared by normal methods, and the usual procedures for treating spectrograms quantitatively are applied.

This method can be used for quantitative and comparative diagnostic purposes. Spectrograms obtained from a synthetic standard containing 0·3 mg. of potassium, 0·3 mg. of calcium, 0·06 mg. of magnesium, 0·06 mg. of sodium, 0·006 mg. of iron, and 0·001 mg. of manganese are reproduced to illustrate the sensitivity of the method. D. A. P.

Identification of Organic Compounds by Use of Chromium Target X-Ray Diffraction Powder Patterns. F. W. Matthews and J. H. Michell (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 662–665)—The identification of organic compounds by X-ray diffraction methods is made difficult by the complexity of the powder pattern. Greater dispersion of the pattern should improve this condition, and this can be accomplished by the use of longer wave-length radiation and/or larger camera diameter. The latter necessitates a refinement of the camera collimator system and would result in longer exposure times. In the work now described, the former method has been used and the patterns of the anilides of the normal saturated fatty acids (C_1 to C_{18}) are shown to be sufficiently characteristic to enable individual identification.

Details of X-ray apparatus, technique, and preparation of the anilides are given.

Powder diffraction data—Lattice spacings and intensities are given in the conventional way for the samples examined. The diffraction data show that the patterns of the anilides of the normal acids C_1 to C_8 are readily distinguishable. The patterns of the even numbers of the series C_8 to C_{18} exhibit marked similarity, suggesting a uniformity of crystal structure, but each pattern can be distinguished; in particular, the longest spacing for each shows a stepwise change with increasing chain length of the acid. Two acids with an odd number of carbon atoms greater than C_8 were also examined and a separate "isostructural" series is indicated for these compounds.

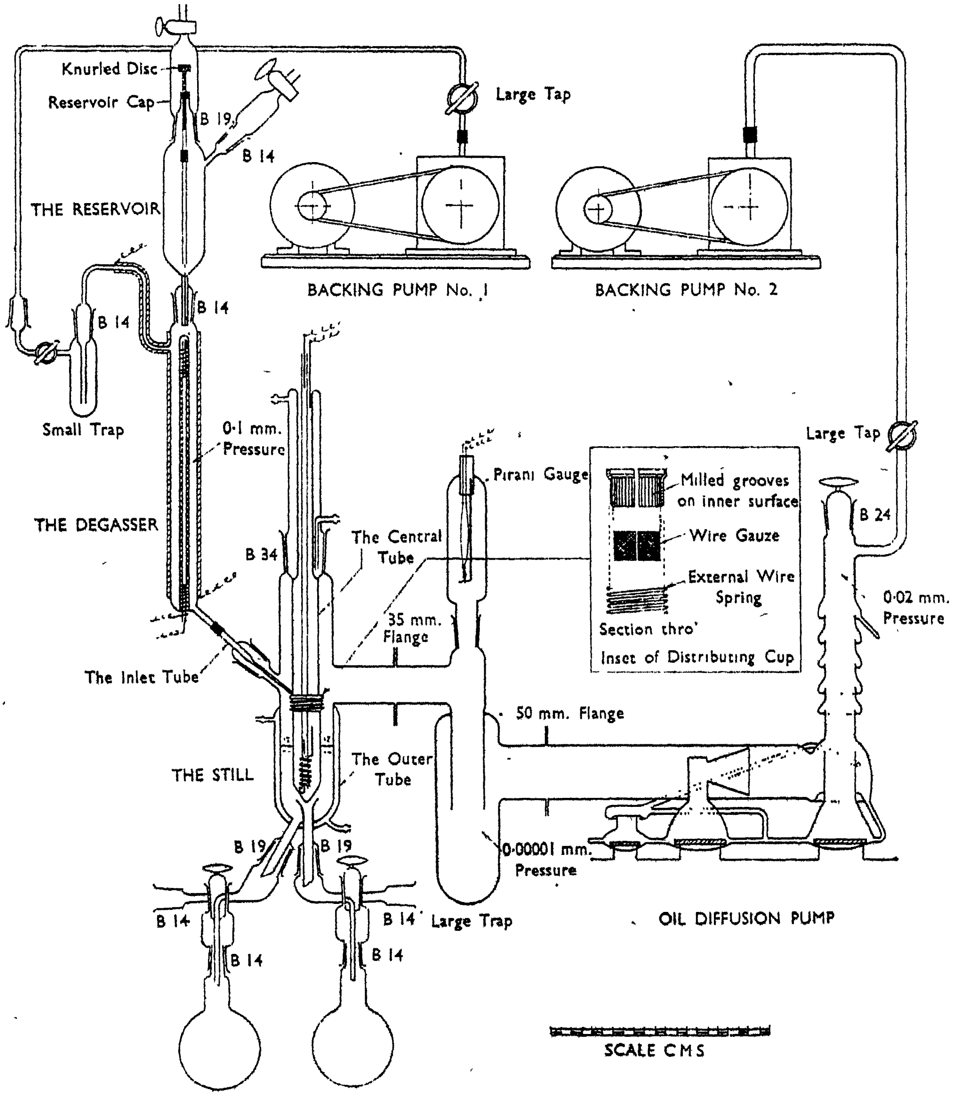
The patterns of the anilides of the *normal* and *iso* isomers of butyric, valeric, and caproic acids could be readily distinguished. Four structural isomers of the C_5 acid were examined; *viz.*, *n*-valeric, *iso*-valeric, α-methylbutyric, and pivalic acids; the anilides of these could also be readily distinguished.

No evidence of polymorphic changes was observed in these compounds, even after several months. All samples were crystallised from solvents at room temperature, and these conditions would tend to give a product stable at room temperature.

Extreme purity of sample is not necessary in obtaining distinctive diffraction patterns.

E. G. S.

Simple Laboratory Falling-film Molecular Still. E. H. Farmer and D. A. Sutton (*J. Soc. Chem. Ind.*, 1946, **65**, 164–166)—A robust and easily operated, falling-film, molecular still in glass is described. It is of non-cyclic type, and has a wide range of usefulness for laboratory work in which moderate quantities of material are to be dealt with. Viscous liquids that do not flow, or mobile fluids that do not spread evenly over the glass distilling surface at the desired distillation temperature, are unsuitable for use in the apparatus. The assembly is made as completely as possible in Pyrex glass whence pin-holes may be readily detected by use of a high-frequency discharge, and the still may be left evacuated indefinitely without leakage. Backing-pump No. 1 should be capable of maintaining a pressure of about 0·1 mm. of mercury in the degasser, while No. 2 should maintain a pressure of 0·02 mm. in the oil-diffusion pump, trap, and still. The thick rubber bungs connecting the metal nozzles of the pumps with the apparatus should be waxed over with "Apiezon Wax W." A two-jet Hickman all-glass diffusion pump with a nominal pumping speed of 12 litres per sec. at 10^{-4} mm. is employed: a three-jet pump of the same type is recommended. The large trap is immersed in a Dewar vessel containing liquid air during use, and the gauge attached to it is a Pirani instrument having a range of 0·5 mm. to 0·0001 mm. This may be replaced with advantage by a Philip's Ionisation gauge reading to 10^{-6} mm. The still consists essentially of two concentric glass tubes, the inner one comprising a reflux condenser and a heated distilling tube joined in one piece. The source of heat is a boiling solvent or suitable mixture of solvents electrically heated, the condenser serving to return the solvent to the tube. The distilling (outer) surface of this tube may be roughened to aid the distribution of the distilland over its surface. The even formation of a thin film of the distilland over the distilling surface is achieved by the distributing cup shown separated and in section in the inset diagram. It consists of a stainless steel cup with many grooves, either straight or spirally disposed, milled into its inner surface. This fits over a piece of stainless steel wire gauze of 50- to 150-mesh, and is made to spring tightly over the distilling tube and gauze, and kept in place by a strong external wire spring. The



outer tube of the still has a water-jacket, but this is unnecessary for distilling temperatures over 125° C. The degasser consists of an electrically heated outer tube to the base of which is sealed a smaller concentric tube also electrically heated by an internal heating-coil. The crude distilland drops from the reservoir on to the top of the inner tube at a rate readily controlled by turning the knurled disc, thereby altering the length of stout tungsten wire partially obstructing the glass capillary connecting the reservoir to the degasser as desired. The high vacuum space inside the still is effectively sealed from the relatively low vacuum in the degasser by the distilland flowing through a constriction near the end of the inlet tube. Reference to the original paper must be made for complete details. J. A.

Reviews

A NEW NOTATION AND ENUMERATION SYSTEM FOR ORGANIC COMPOUNDS. By G. MALCOLM DYSON. Pp. 63+iv. London: Longmans, Green & Co., Ltd. 1947. Price 7s. 6d.

For the best part of a century the biggest problem confronting organic chemists has been the naming and indexing of the vast number of compounds they have discovered, made, or envisaged. The Geneva system of 1889, modernised in 1930, and the Ring Index arising therefrom, leave many classes of compound out of consideration, often fail to provide a usable name, and have now diverged far from current usage. The complexity of the system has led to grave arrears in the indexing of information and the compilation of reference works.

Dr. Dyson has tackled the problem as Dr. Clarence Smith hoped it would be tackled when he lectured to the Chemical Society in 1936, by making a fresh start and discarding the old terminology. The basis of the new system is described elsewhere in this issue of THE ANALYST; it will be seen that the ideograph of each compound is dissected in a logical and straightforward manner, each part or attribute of it being successively delineated by a combination of letters, numbers, and symbols unique for each substance. No longer is there confusion in the numbering of rings, or chains, or carbon atoms; no longer may one use alternatively numbers or Greek letters. The cipher can be readily and strictly classified and indexed; "trivial" names engendered by the complexities of the old systematic nomenclature need no more appear in indexes; and there is a strong basis for believing that within a reasonable time it will be possible to produce a lexicon of organic chemistry only a few months behind the then-current wave-front of publication.

Such a development in the history of organic chemistry is obviously of the utmost importance. Dr. Dyson truly merits the profound gratitude of his fellow chemists for his remarkable work, so ably described in this booklet, of replacing the turgid complexities of the Geneva system by a design of modern elegance, precision, and proportion most fitting to the times. K. A. WILLIAMS

SUPPLY OF LABORATORY APPARATUS AND CHEMICALS

THE Council of the Society will be pleased if any member who is experiencing difficulty in obtaining delivery of laboratory apparatus or chemicals will send details of such difficulties to the Hon. Secretary, Mr. K. A. Williams, 6, Milner Street, London, S.W.3, in order that specific instances may be brought to the notice of the Controlling Authority.

The Council would also be pleased to receive the views of any member on his requirements of apparatus and chemicals for the coming year.

MICROCHEMISTRY GROUP

A MEETING of the Group will be held jointly with the South Yorks. Section of the Royal Institute of Chemistry and the Sheffield Metallurgical Association on Friday, May 16th, 1947, at Sheffield. There will be a visit to the Bragg Laboratory of the Admiralty in the afternoon. At the evening meeting, which will begin at 5.45 p.m. in the Chemical Department of the University, the following papers will be read:

"The Determination of Carbon, Hydrogen, and Nitrogen in Aliphatic Nitro Compounds," by A. E. Heron.

"A Review of Micro Methods for the Determination of Oxygen in Organic Compounds," by C. E. Spooner.

"A New Spot-test for the Detection of Sulphites and Sulphur Dioxide," by R. Belcher and G. Ingram.

"The Microchemical Analysis of Aluminium Base Alloys," by J. Townend and C. Whalley.

MAY, 1947 24 JUL 1947 Vol. 72, No. 854

THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

An Ordinary Meeting of the Society was held at 6 p.m. on Wednesday, April 2nd, at the Chemical Society's Rooms, Burlington House, London, W.1. The chair was taken by the President, Mr. Lewis Eynon. The following papers were presented and discussed: "A Note on the Chapman and McFarlane Method for the Estimation of Reducing Groups in Milk Powder," by C. H. Lea, D.Sc., F.R.I.C.; "The Determination of Carotene in Dried Grass," by W. A. G. Nelson, B.Sc.,Ph.D.

NEW MEMBERS

William James Carpenter, A.R.I.C.; Henry Edward Coomber, B.Sc. (Lond.); Charles Donald Cook, A.R.I.C.; John Ruff Gwilt, A.R.I.C.; George Edmund Holmes, M.A. (Cantab.); Harold Thomas Islip, B.Sc. (Lond.), F.R.I.C.; Alexander Johnston, B.Sc. (Glas.); Henry Kenneth Lawton, B.Sc., Ph.D. (Liv.), F.R.I.C.; Leopold Ferdinand Levy, M.Sc. (Witwatersrand), Ph.D. (Lond.); Colin Marsden, B.Sc., Ph.D.; Harry Polkinhorne, B.Sc. (Lond.), A.R.I.C.; Arthur Henry Snowden, A.R.I.C., Ph.C.; John Ivor Webb, B.Sc., Ph.D. (Birm.), F.R.I.C.; William Sydney Durham Wise, B.Sc. (Bris.).

DEATH

We regret to have to record the death of

Sura Rajagopal Naidu

SCOTTISH SECTION

The twelfth Annual General Meeting of the Scottish Section was held in Glasgow on January 15th, 1947. The following is the list of office bearers elected for the year.

Chairman—Dr. H. Dryerre. *Vice-Chairman*—Dr. J. Sword. *Hon. Secretary and Treasurer*—R. S. Watson. *Other Members of Committee*—A. R. Jamieson, H. C. Moir, J. Sandilands, A. M. Smith, R. G. Thin and S. L. Tompsett. *Hon. Auditors*—A. R. Campbell and W. M. Cameron.

MICROCHEMISTRY GROUP

At the Annual General Meeting of the Group, held on Friday, January 31st, 1947, at the Sir John Cass Technical Institute, London, E.C.3, Mr. Norman Strafford was elected Chairman in place of Professor H. V. A. Briscoe, who had completed his term of office, Dr. Janet Matthews was elected Vice-Chairman and Professor Briscoe, Mr. J. T. Stock and Mr. E. J. Vaughan were elected Ordinary Members of the Committee in place of Miss I. H. Hadfield, Dr. Janet Matthews and Dr. G. H. Wyatt who, having served for two years, were due to retire.

After the Annual General Meeting the following papers were read and discussed: "A Review of Electrolytic Methods of Microchemical Analysis," by A. J. Lindsey; "The Oxidation of Nitrogen during the Micro-combustion of Organic Substances," by A. E. Heron (see this Vol., p. 142); "The Micro Separation of Gums from Crystals," by A. L. Bacharach; "The Microchemical Determination of Molybdenum in Steel," by J. E. Wells and R. Pemberton (see this Vol., p. 185).

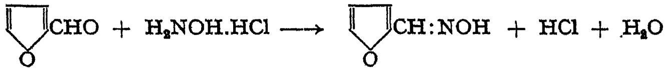
Linlithgow Library.
Imperial Agricultural Research Institute.

Some Analytical Methods Applicable to Furan and its Derivatives

BY F. G. ANGELL

IN recent years attention has been focussed on the utilisation of waste vegetable material as a potential source of commercial chemicals, notably furfural and other furan derivatives. Little information exists in the literature concerning analytical methods applicable to such compounds, and the present paper deals with quantitative methods that have been employed in the examination of a large range of furan derivatives prepared in the Research Department of Imperial Chemical Industries, Ltd. (Billingham Division), including furfural, furfuryl alcohol, tetrahydrofurfuryl alcohol, furan, sylvan, tetrahydrofuran and tetrahydrosylvan. These methods may be classified under three headings, *viz.*, Oximation, Absorptiometric and Bromination.

1. OXIMATION—

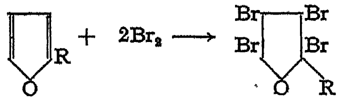


The extent of the reaction is ascertained by titration of the hydrochloric acid liberated from neutralised hydroxylamine hydrochloride and the method is in general limited to the estimation of large amounts of furfural. Various furan compounds (*e.g.*, furfuryl alcohol), when warmed with the reagent, undergo ring opening with the production of aldehydes, and this behaviour seriously limits the applicability of the method.

2. ABSORPTIOMETRIC—

The production of a red colour by mixing furfural and aniline acetate has been recognised for a long time as a specific qualitative test for the former compound (and thus for pentosans). By working under carefully controlled conditions on the basis of this test,[2] an absorptiometric method has been developed and is of considerable utility in the determination of subordinate amounts of furfural present as impurity in other furan compounds.

3. BROMINATION—



"Pyridine sulphate bromide" has been found to effect complete saturation of the ring with such compounds as furfuryl alcohol, furan, and sylvan. Little, if any, substitution occurs, for the corresponding tetrahydro compounds are practically inert towards the reagent. It is therefore of great utility in the analysis of such compounds. Certain furan derivatives, *e.g.*, furoic acid and furfural, are unaffected by this reagent although they are attacked by acidified potassium bromide - bromate solution. Furoic nitrile exhibits unique behaviour in that it is inert towards all ordinary bromination reagents.

EXPERIMENTAL

1. OXIMATION OF FURFURAL

Redistilled furfural (b.p. 162·0° to 162·2° C.) which was pale straw yellow in colour and neutral towards methyl yellow was used in investigating the method. The results in Table I, obtained by the method described below, indicated that oximation did not proceed further than 98·0 per cent. even if the sample was left in contact with the reagent for 1 hour. No increased precision resulted from the use of larger weights of sample involving correspondingly larger titrations. Examination of some solutions whose concentrations were not known to the analyst (Nos. 4 to 6 in Table I), on these lines, confirmed that the reaction was not quite stoichiometric. Owing to the intense buffering action of the excess of hydroxylamine hydrochloride, titrations with 0·1 *N* sodium hydroxide are not recommended, as the end-points so obtained are too indefinite, and in such circumstances larger quantities of sample should be used for analysis or the furfural determined by the aniline acetate method.

When applied to the determination of furfural in mixtures containing furfuryl alcohol and the like it is essential that the oximation be carried out in the cold, for on being warmed furfuryl alcohol readily undergoes ring opening with the production of various compounds containing a carbonyl group. Although these compounds will react with hydroxylamine hydrochloride, this reaction is not suitable for the accurate determination of furfuryl alcohol, because variable quantities of dark-coloured polymerised products are often also formed.

TABLE I

Expt. No.	Wt. of sample present, mg.	0·5 *N* NaOH required, ml.	Wt. of furfural found, mg.	Percentage oximation
1	528·1	10·75	516·0	97·6
2*	630·4	12·90	619·2	98·2
3	624·8	12·80	614·4	98·3
4	492·2	10·10	484·8	98·5
5	560·9	11·45	549·6	98·0
6	422·0	8·68	416·6	98·7
			Mean	.. 98·2 ± 0·6

* 1 hr. standing.

METHOD—

Reagents—

(1) A 5 per cent. w/v solution of hydroxylamine hydrochloride in water, neutralised with 0·5 *N* sodium hydroxide to the orange tint of methyl yellow.
(2) Refined methanol (of low aldehyde content).
(3) Indicator—a 0·1 per cent. solution of methyl yellow in methanol.
(4) Sodium hydroxide solution, 0·5 *N*, free from carbon dioxide.

Procedure—Weigh 0·5 to 0·7 g. of the sample by difference, from a weighing pipette, into a 250-ml. beaker containing 15 ml. of neutralised hydroxylamine hydrochloride solution (50 per cent. excess) and 15 ml. of methanol. After half an hour titrate the liberated hydrochloric acid in good daylight with 0·5 *N* sodium hydroxide to the orange tint of the indicator, using a blank solution containing the same volume of reagents to assist in judging the end-point.

2. ABSORPTIOMETRIC DETERMINATION OF FURFURAL

Determination of subordinate quantities of furfural (about 1 mg.) have often been conducted by matching the red colour produced with aniline acetate against a series of standards diluted to a convenient volume with methanol in Nessler cylinders. Preliminary experiments indicated that in the presence of methanol the optical extinctions measured on the Spekker absorptiometer with an appropriate filter diminished rapidly on standing, and moreover no advantage was gained by substituting aniline hydrochloride for the acetate. Of the various methods proposed in the literature for developing this test on an absorptiometric basis the procedure of Stillings and Browning,[1] as modified by Duncan,[2] seemed the most attractive. The latter author recommended a mixture of disodium phosphate and oxalic acid as a stabiliser, but it was found that disodium phosphate alone was quite satisfactory for this purpose. Further, it seemed desirable to avoid using oxalic acid, because of the sparing solubility of

TABLE II

SOLUTION CONTAINED 0·554 MG. OF FURFURAL—EXTINCTIONS IN 1-CM. CELL ($E_{1\,cm.}$) FOR Hg LINE 5461A (CALOREX 503 AND ILFORD 605 FILTERS)

2·5 g. of aniline + 25 ml. of glacial acetic acid and 1 g. of $Na_2HPO_4.12H_2O$ diluted to 100 ml. with water.		2·5 g. of aniline + 10 ml. of glacial acetic acid + 1 g. of $Na_2HPO_4.12H_2O$ diluted to 100 ml. with water	
Time (mins.)	$E_{1\,cm.}$	Time (mins.)	$E_{1\,cm.}$
30	0·272	5	0·014
45	0·303	15	0·077
60	0·330	30	0·169
75	0·354	45	0·159
90	0·353	60	0·147
105	0·354		
150	0·337		

sodium hydrogen oxalate. A large volume of the mixed reagent prepared according to the directions of Duncan deposited, on standing, a considerable quantity of a crystalline precipitate, presumably this salt.

The effective time for the maximum development of the colour with aniline acetate and disodium phosphate, the period of stability, and the effect of using lesser quantities of acetic acid were next investigated. As will be seen from Table II, with less acetic acid the colour was not so fully developed and faded more rapidly, but in presence of 1 g. $Na_2HPO_4.12H_2O$ and 25 ml. of glacial acetic acid containing 2·5 g. of freshly distilled aniline the extinction was at its maximum between 75 and 105 minutes, after which time it gradually diminished.

METHOD—

Reagents—

(1) A 10 per cent. w/v solution of freshly distilled aniline in glacial acetic acid (freshly diluted each day).

(2) A 10 per cent. w/v solution of $Na_2HPO_4.12H_2O$ in water.

Procedure—Adjust the temperature of all solutions to 20 ± 0·5° C. before mixing. Measure an aliquot of the sample solution, containing 0·2 to 1·0 mg. of furfural; it should be neutral to phenolphthalein. Add 10 ml. of 10 per cent. $Na_2HPO_4.12H_2O$ solution, dilute to 70 ml. with water, and finally add 25 ml. of aniline acetate reagent and adjust the volume to 100 ml. Mix well and immediately transfer to a dark cupboard. At the same time prepare a blank solution in an identical manner. After the expiration of 1¼ hrs. measure the extinction in a 1-cm. cell for the Hg line 5461A, using Calorex 503 and Ilford 605 filters, with the blank solution in No. 2 cell. The results given in Table III were obtained in this manner; from them was derived the relation:

$E_{1\,cm.} \times 1{\cdot}50 \equiv$ mg. of furfural per 100 ml. of solutions for values of $E_{1\,cm.}$ below 0·7.

TABLE III

Wt. of furfural, mg.	$E_{1\,cm.}$ 5461 A.	Factor
0·156	0·100	1·56
0·469	0·314	1·49
0·782	0·528	1·48
0·940	0·634	1·49
1·096	0·726	1·51
1·253	0·781	1·61
1·409	0·824	1·71

The colour is virtually specific for furfural and unaffected by acetone, furfuryl alcohol or tetrahydrofurfuryl alcohol. Table IV gives the results obtained on some typical products containing small quantities of furfural.

TABLE IV

Description of sample	Furfural % (absorptiometric)	Furfural % (by oximation)
Distillate obtained by digestion of waste vegetable material (containing ketones, etc.)	1·50	1·58
Ditto	1·48	1·54
Redistilled tetrahydrofurfuryl alcohol (containing 1·3% of furfuryl alcohol)	0·022	Detected <0·1
Ditto (containing 0·4% of furfuryl alcohol)	Not detected <0·01	—
Furfuryl alcohol: Sample A	0·66	0·64
„ B	0·69	0·61
„ C	1·10	1·11

3. BROMINATION

(1) USE OF PYRIDINE SULPHATE BROMIDE—

This reagent* has received considerable application[3,4,5,6] in the determination of the unsaturation of hydrocarbons and is stated to produce very little substitution. Thus Fitelson[7] employed it for the determination of squalene, and Wilson and Nisbet[8] applied it in the analysis of various shale oil fractions. With compounds containing a furan ring, complete saturation usually takes place in accordance with the equation given on p. 178. Further work revealed that the nature of the substituent R exerted a very marked effect on the behaviour of the reagent. Thus if R = H, CH_3 or CH_2OH saturation of the ring readily occurred, but if R = CHO, COOH or CN little or no reaction was observed. Moreover, practically no reaction occurred with the corresponding tetrahydro compounds where R = H, CH_3 or CH_2OH, thus affording strong evidence that but little substitution takes place. This reagent proved to be a powerful weapon with which to attack the problem of the analysis of furan compounds, and with its aid methods have been devised for the determination of furfuryl alcohol in admixture with furfural and tetrahydrofurfuryl alcohol and for the determination of furan and sylvan in presence of the corresponding tetrahydro compounds. Details of the method used are given later (p. 182).

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* For preparation of this reagent see below, p. 182.

(I) DETERMINATION OF FURFURYL ALCOHOL—A sample of refined furfuryl alcohol (b.p. 78° to 80° C./20 mm., D_4^{20} 1·1337, n_D^{20} 1·4870, furfural 0·24 per cent.) was analysed by the method described below and the furfuryl alcohol content calculated on the assumption that complete saturation of the ring occurred.

Time of contact with reagent	Furfuryl alcohol found, %
15 mins.	93·0
30 ,,	97·0
60 ,,	100·0, 100·0, 100·0, 100·6, 99·3

Thus quantitative bromination occurred in 1 hour, while furfural gave a "bromine value" of 30 to 35 g. of Br per 100 g. (equivalent to 10 per cent. of furfuryl alcohol) and tetrahydrofurfuryl alcohol a value of less than 1 (equivalent to $<0·3$ per cent. of furfuryl alcohol) under similar conditions. Mixtures of furfuryl alcohol, tetrahydrofurfuryl alcohol and furfural, whose composition was unknown to the analyst, were examined by the method detailed below, with the results given in Table V.

TABLE V

Furfural present, %	Tetrahydrofurfuryl alcohol present, %	Furfuryl alcohol present, %	Furfuryl alcohol found, %	Deviation % of mixture
0	0	100·0	98·4	−1·6
7·1	7·6	85·3	84·4	−0·9
6·0	7·3	86·7	85·9	−0·8
3·5	32·6	63·9	63·1	−0·8
31·6	40·4	28·0	28·6	+0·6
48·6	25·0	26·4	26·8	+0·4
62·2	20·9	16·9	18·7	+1·8
5·5	78·9	15·6	16·1	+0·5
68·9	19·8	11·3	11·8	+0·5
81·2	10·5	8·3	10·0	+1·7
5·4	88·9	5·7	5·9	+0·2
90·0	5·2	4·8	5·9	+1·1

Thus, provided that the furfural content of such a ternary mixture does not exceed 20 per cent., furfuryl alcohol can be determined to within ±1 per cent. of the true value and the method is obviously applicable to the determination of small amounts of furfuryl alcohol present as impurity in tetrahydrofurfuryl alcohol.

When the method was applied to the analysis of technically pure furfuryl alcohol, inconsistencies appeared in the results and were ultimately traced to the ease with which furfuryl alcohol and some other furan compounds undergo polymerisation. It was found that the furfuryl alcohol had changed after storage for several weeks. Vacuum distillation in a current of carbon dioxide yielded 89·6 per cent. of distillate (b.p. 87° C./20 mm.), which proved to be furfuryl alcohol of purity 99 per cent., 3·2 per cent. of distillate consisting of less pure furfuryl alcohol, and 5·8 per cent. of dark brown sticky residue which became a brittle solid on cooling to room temperature. The results of this experiment are summarised in Table VI.

That the low and inconsistent results were attributable to the presence of polymerised substances was finally demonstrated by addition of varying quantities of "heavy ends" to a redistilled sample; in all instances a rapid diminution in bromine value resulted. Thus addition of an equal weight of heavy ends to a sample showing 92·0 per cent. of furfuryl alcohol gave a result of 45·7 per cent. of furfuryl alcohol. The rapidity with which polymerisation took place is illustrated by the observation that when 10 g. of freshly distilled sample contained in a platinum dish were evaporated to dryness on a steam bath 2·1 per cent. of hard non-volatile residue remained. Therefore, all subsequent analyses by bromination methods on furan and its derivatives were conducted on freshly distilled samples.

TABLE VI

Fraction No.	% of original	% Furfuryl alcohol (pyridine sulphate bromide method)
1	61·2	99·5
2	28·4	98·7
3	3·2	67·7
4 (Residue)	5·3	
Loss	1·9	

(II) DETERMINATION OF SYLVAN AND FURAN—In an identical manner the behaviour of sylvan, tetrahydrosylvan, furan, tetrahydrofuran, furoic acid and furoic nitrile were investigated, freshly distilled material being used for the liquid substances. The results obtained are indicated in Table VII.

TABLE VII

Compound	Wt. taken, mg.	Bromine value	Apparent % of sylvan or furan
Sylvan Theoretical bromine value 391	190	396	101·4 sylvan
	19·9	386	98·9 ,,
	27·89	394	101·0
	24·13	392	100·6
Tetrahydrosylvan	770	12	3·1
,,	179	11·6	3·0
,,	60·5	4·6	1·2
Furan Theoretical bromine value 470	20·369	465	98·9 furan
	15·437	478	101·7
Tetrahydrofuran: Sample A	1780	<0·2	<0·1
,, B	444	4·7	1·0
,, C	443	5·7	1·2
,, D	443	10·0	2·1
Furoic acid	260	3·4	—
,, nitrile	300	3·9	—

The results in Table VII indicate that furan and sylvan react quantitatively with the reagent, but this exerts only a slight action on tetrahydrosylvan, possibly attributable to substitution. With tetrahydrofuran, however, independent confirmation of the presence of furan (and possibly sylvan) as impurities has been obtained from phase-rule studies of the system water, tetrahydrofuran and furan (unpublished work).[9] Thus sample A was specially purified by batch fractionation in a high efficiency column and was perfectly miscible with water in all proportions. On the other hand B, C, and D, were miscible with water only over a limited range of concentrations, and phase-rule work indicated about 2 per cent. of furan and sylvan in samples B and C and about 4 per cent. in sample D.

METHOD USED FOR THE DETERMINATION OF FURFURYL ALCOHOL, SYLVAN, AND FURAN—

Reagents—

(1) Pyridine sulphate bromide reagent (approx. 0·05 N with respect to bromine). Into each of 3 dry 500-ml. conical flasks measure 40 ml. of glacial acetic acid. Cool, and add to the first 16·3 ml. of pure pyridine and to the second 10·9 ml. of sulphuric acid (sp.gr. 1·84). When cold, combine these two solutions, with further cooling, and to the third flask add 3·2 ml. of bromine. Finally, add the contents of the third flask to the previous mixed solution, dilute to 2 litres with glacial acetic acid and mix thoroughly by shaking.

(2) A 10 per cent. w/v potassium iodide solution.

(3) Sodium thiosulphate solution, 0·1 N.

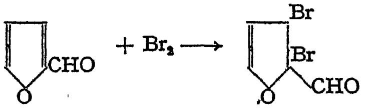
For unsaturated compounds introduce into a tared oleum bulb an appropriate weight of sample (20 to 25 mg. of furan or sylvan, 30–35 mg. of furfuryl alcohol), seal, and reweigh on a micro balance. Alternatively use larger quantities of sample, dilute to a convenient bulk with glacial acetic acid in a volumetric flask and withdraw an appropriate aliquot for analysis. With saturated compounds use any suitable larger quantity of material.

Procedure—Pipette 50 ml. of the reagent (1) into a 200-ml. stoppered "iodine" flask, introduce the sample *via* the sealed oleum bulb or otherwise, break the bulk carefully under the surface of the solution by means of a glass rod and wash down the rod with a few ml. of glacial acetic acid. Promptly replace the stopper and seal the neck of the flask with 5 ml. of potassium iodide solution. Allow to stand in the dark for 1 hour, together with a "blank" on 50 ml. of the reagent. Release the stopper carefully, allowing the potassium iodide solution to flow into the flask so that no bromine is lost. Add 10 ml. of potassium iodide solution, shake well and titrate the liberated iodine with 0·1 *N* thiosulphate, adding a little starch solution when the titrated solution becomes pale yellow. Frequently the titrated solution is pale brown at the end-point, but no difficulty is experienced in recognising the disappearance of the starch iodide colour.

If B = bromine value = g. of bromine absorbed per 100 g. of sample,
percentage of furfuryl alcohol = 100B/325
,, ,, furan = 100B/470.
,, ,, sylvan = 100B/391.

(2) USE OF ACIDIFIED POTASSIUM BROMIDE - BROMATE SOLUTION—

It has been stated by Hughes and Acree[10] that if furfural be treated with acidified bromide - bromate solution at 0° C. for 10 minutes, only one double bond is attacked, in accordance with the equation:



This procedure was investigated, both the bromide - bromate solution and the 5 per cent. hydrochloric acid used for acidification being cooled in an ice-bath at 0° C. for ½ hr. preceding the bromination tests. With the same sample of redistilled furfural (assaying 98·2 per cent. by aqueous oximation) the following results were obtained (Table VIII).

TABLE VIII

TEST SOLUTION CONTAINED 30·9 MG. OF FURFURAL

Time of bromination, mins.	Furfural found, mg.	Apparent % furfural
8	27·4	90·9
10	29·1	94·2
10	28·6	92·6
15	31·9	103·1

These results indicate that bromination for 10 minutes does not proceed beyond 94 per cent. of completion, whereas with 15 minutes reaction time bromination exceeds 100 per cent. on the basis of the above equation. Moreover, the starch iodide end-point of the subsequent titration is frequently fugitive and may well lead to even greater divergencies than result from slight variation in the time of bromination. This method does not appear to have any advantages over the oximation procedure, and in general it seems undesirable to adopt as a standard procedure any method in which the reproducibility of the results clearly depends on a careful counterbalancing of opposing sources of error.

However, acidified bromide - bromate solution, with addition of methanol as a solvent for the compound, has been applied to the determination of furoic acid alone or in admixture with tetrahydrofuroic acid. Addition of increasing quantities of methanol to the blank solution diminished its titre, but provided that at least 25 per cent. by volume of methanol was present the blank titration was unaffected. As solutions of suitable concentrations of furoic acid in 25 per cent. methanol could be readily prepared, the method was standardised on this basis. The bromination was allowed to proceed for 15 minutes at room temperature, with the usual Koppeschaar technique. Results obtained for furoic acid alone and in mixtures with tetrahydrofuroic acid whose composition was unknown to the analyst are shown in Table IX. Tetrahydrofuroic acid alone gave a bromine value equivalent to 1 per cent. of furoic acid.

TABLE IX

Nature of sample	Furoic acid % found
Furoic acid	97·5
" "	98·0
" "	99·4
Mixture containing 9·8% furoic acid ..	10·2
" " 48·1% " " ..	48·2
" " 76·0% " " ..	77·1

Furoic nitrile was unaffected by this reagent (bromine value = 2) and towards Kaufmann's reagent (bromine + sodium bromide in methanol) it was equally inactive, yielding a bromine value of 3 to 3·5. That the impure nitrile available was an unsaturated compound was confirmed by hydrolysis with dilute sulphuric acid and extraction with ether, when an acid with m.p. 123° to 125° C. was obtained. (Furoic acid has m.p. 130° to 131° C., and tetrahydrofuroic acid is a liquid.) Thus, of all the furan derivatives investigated, furoic nitrile is unique in its inertness towards bromination agents.

SUMMARY—

Existing methods for the analytical examination of furan derivatives have been critically examined and, where necessary, improved.

1. Furfural can be determined by aqueous oximation at room temperature, although the reaction is not quite stoichiometric. Small amounts of furfural should be determined absorptiometrically, using the aniline acetate method.

2. Pyridine sulphate bromide is the most useful reagent for the determination of unsaturation in the furan series. Saturation of both double bonds occurs with furan, sylvan, and furfuryl alcohol, and substitution reactions with the corresponding tetrahydro compounds are negligible. On the other hand, furfural, furoic acid and furoic nitrile are but little attacked by this reagent.

3. Acidified potassium bromide - bromate reagent finds limited application as a brominating reagent for furoic acid, but with furfural only approximately quantitative results are obtained.

In conclusion, I wish to thank Mr. W. Hutchinson for the experimental data on the method employed for furoic acid.

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Erratum.—December issue, 1946, p. 555, line 17 from the bottom: *For* "nickel" *read* "nickelous."

The Microchemical Determination of Molybdenum in Steel

By J. E. WELLS and R. PEMBERTON

(Read at the Annual General Meeting of the Microchemistry Group, January 31, 1947)

In the course of recent investigations it became necessary to determine molybdenum in small quantities of steel. A review of available methods led to the conclusion that the reactions involved were not of sufficient sensitivity for the work in hand. It was known, however, that molybdenum would give a green precipitate with toluene-3:4-dithiol in acid solution,[1] and work was initiated to determine the suitability of the reaction for quantitative determinations.

Preliminary work indicated that the green molybdenum compound produced in cold hydrochloric acid solution could be extracted with amyl acetate. The amyl acetate extract could be rendered clear and free from turbidity by washing with concentrated hydrochloric acid, and the absorptiometric evaluation of its colour bore a linear relationship to the molybdenum concentration. Under these conditions, that is, in cold hydrochloric acid solution, and in absence of stannous chloride, any tungsten present reacted only slowly, the reaction rate decreasing with decreasing acid concentration.

These results suggested the possibility that under suitable conditions interference by tungsten might be eliminated, and a determination of molybdenum carried out. Investigations were, therefore, put in hand to determine these conditions.

EXPERIMENTAL

For the purpose of these experiments, "synthetic" steel solutions were made up from a solution of pure iron in sulphuric acid. Aliquots equivalent to four milligrams of metal were used as a test basis, and other elements added in the form of standardised solutions.

Preparation of pure iron solution—One gram of pure iron was dissolved in 18 ml. of 10 per cent. sulphuric acid. After oxidation with the minimum amount of nitric acid, the solution was boiled down almost to dryness, taken up with water, and made up to one litre in a graduated flask. Aliquots of this solution containing 4 mg. of iron were then taken per test, each containing the equivalent of 0·0012 ml. of free sulphuric acid.

Effect of Hydrochloric acid concentration on the extraction of the coloured molybdenum complex—Solutions representing 4-mg. samples of a synthetic 0·70 per cent. molybdenum steel to which the equivalent of 0·05 ml. of concentrated sulphuric acid had been added, were boiled down to fuming, and the fumed liquids were taken up in 3-ml. portions of hydrochloric acid, the concentration of which was varied from sample to sample. The solutions were then cooled, and 3 ml. of a 1 per cent. solution of toluene dithiol in amyl acetate were added to each. After standing for varying periods of time with occasional shaking, the amyl acetate layer of each was separated, washed with 3 ml. of concentrated hydrochloric acid, transferred to a 10-ml. graduated flask and made up to the calibration mark with amyl acetate. The absorption was then measured on the Spekker photo-electric absorptiometer, using Calorex heat-resisting filters H503, spectrum red filters 607, and a 1-cm. micro cell.

The results obtained, in Spekker units, were as follows:

Sp.gr. of hydrochloric acid at 15° C.	Standing time before separation (minutes)				
	5	10	20	30	40
1·021	—	—	0·090	—	0·120
1·043	—	0·130	—	—	0·190
1·051	0·295	0·330	—	—	0·350
1·061	0·530	0·560	0·560	0·560	0·560
1·075	0·555	0·560	0·560	0·555	0·560
1·082	0·560	0·560	0·565	0·560	0·560
1·097	0·565	0·560	—	0·560	0·560
1·182	0·555	0·560	0·560	0·565	0·560

These figures show uniform results for all hydrochloric acid concentrations of specific gravity between 1·075 and 1·182, and for all time intervals tested over this range of acidity. Acid of specific gravity 1·075 was selected for further work, as preliminary experiments, on the effect of tungsten, had already indicated that this acidity would achieve the best compromise in relation to the suppression of the corresponding dithiol tungsten complex.

In such acid concentration, any tungsten present in the steel would tend to separate as tungstic acid, possibly carrying with it some molybdenum, and thus giving low results. Further experiments were, therefore, carried out in order to determine the effect of phosphoric acid on the development of the molybdenum colour. For convenience the phosphoric acid was added as "Spekker" acid (15 per cent. sulphuric acid, 15 per cent. phosphoric acid). A series of test solutions, each containing the equivalent of 4-mg. of synthetic 0·70 per cent. molybdenum steel, were prepared, and different amounts of "Spekker" acid were added to each. The solutions were evaporated to fuming, taken up in 3 ml. of hydrochloric acid of sp.gr. 1·075 and cooled; the dithiol was added, and the separations were carried out as before.

Amount of "Spekker" acid used, ml. ..	nil	0·25	0·50	0·75	1·00
"Spekker" reading	0·555	0·560	0·560	0·565	0·560

These results show that the amounts of "Spekker" acid in the range investigated have no effect on the intensity of the colour due to molybdenum. All further work was therefore carried out in the presence of 0·5 ml. of "Spekker" acid, as this amount was found effectively to prevent the hydrolysis of up to 16 per cent. of tungsten on the sample weights used.

Effect of acid concentration on the formation of the tungsten complex—Most previous work done with toluene-3:4-dithiol has been concerned chiefly with its use for the detection and estimation of tungsten[2,3] and tin.[4] It was, therefore, thought advisable at this stage of the investigation to ascertain the exact conditions necessary for suppression of the reaction with tungsten. An addition equivalent to 4 per cent. of tungsten was therefore made to a number of solution samples of synthetic 0·70 per cent. molybdenum steel, each containing the 0·50 ml. of "Spekker" acid previously decided upon. The samples were then evaporated to fuming, and taken up in 3 ml. of hydrochloric acid solution, the concentration of which was varied from test to test. After the addition of 3 ml. of dithiol solution the amyl acetate layer was separated and its absorption measured as before:

Sp.gr. of hydrochloric acid used	1·064	1·075	1·083	1·097	1·182
"Spekker" reading	0·560	0·565	0·560	0·660	0·805

These readings show that 4 per cent. of tungsten has no effect on the determination of molybdenum provided the reaction is carried out in hydrochloric acid of specific gravity between 1·064 and 1·083. This confirms that the specific gravity previously decided upon, namely, 1·075, is probably the best compromise between the conditions necessary, on the one hand for complete suppression of the tungsten colour, and on the other for complete development of the molybdenum colour.

Effect of dithiol concentrations—The above conditions were applied except for the use of dithiol solutions of differing concentrations, 3 ml. being added in each case. Extractions were made on synthetic solutions of three types of steel containing respectively:

(*a*) 0·70 per cent. of molybdenum.
(*b*) 0·70 „ „ „ „ plus 4·0 per cent. of tungsten.
(*c*) 4·0 „ „ „ „

	Concentration of dithiol solution				
Sample	0·25%	0·50%	1·0%	2·0%	3·5%
	"Spekker" readings				
(*a*) 0·70% Mo	0·515	0·560	0·560	0·565	0·560
0·70% Mo + 4·0% W ..	0·520	0·565	0·565	0·570	0·615
4·0% Mo (0·25 cm. cell) ..	—	0·770	0·775	0·775	0·770

These figures indicate that 3 ml. of 0·50 per cent. dithiol solution would be adequate, but in order to be sure of a safe excess it was decided to use the 1 per cent. solution.

Stability of colour—The amyl acetate solution of the molybdenum complex was found, after separation, to undergo no measurable change in absorption in twenty-four hours.

Effect of temperature—Tests made on synthetic solutions containing tungsten showed that the formation of the tungsten complex is inhibited only under the conditions previously specified, in which the colour development is allowed to proceed at room temperature. For example, the following figures show for two synthetic samples one of which contained the equivalent of 4 per cent. of tungsten, the effect on the results of heating for 15 minutes on the water bath before separation of the amyl acetate extract.

	"Spekker" readings	
Sample contained	Normal treatment	Fifteen mins. on the water-bath
0·70% Mo	0·560	0·570
0·70% Mo + 4·0% W	0·560	0·700

It is clear, therefore, that the selective formation of the molybdenum complex is dependent on the use of a relatively low temperature for colour development.

Effect of other elements—The effect of other elements likely to be found in steel, in the procedure evolved, has been examined. No interference is detected when tungsten is present in amounts below 16 per cent., as shown by the following results on a synthetic 0·70 per cent. molybdenum steel containing variable amounts of tungsten:

Percentage of tungsten	0·0	2·0	4·0	8·0	16·0
"Spekker" reading ..	0·560	0·565	0·560	0·555	0·560

Cobalt, vanadium, tin, lead, niobium, tantalum, manganese, and aluminium do not interfere when present in amounts up to 10 per cent. Nickel and chromium have shown no interference below 25 per cent. Titanium has shown no interference up to 1 per cent., and selenium and silicon none up to 5 per cent.

Iron present in the ferric state is partially extracted by the amyl acetate under the conditions used, and accounts for the small "blank" observed, *i.e.*, 0·01 Spekker unit.

If 10 per cent. of copper is present, the amyl acetate layer becomes cloudy and black, but rapidly clears when washed with the concentrated hydrochloric acid, leaving a faint cloudiness which invariably gives a reading equivalent to 0·015 per cent. of molybdenum.

Zirconium, in amounts up to 10 per cent., gives a pale green colour equivalent to 0·025 per cent. of molybdenum. This colour may be due to impurities in the zirconium used.

We may, therefore, conclude that in practical steel analysis this method is specific for molybdenum.

RECOMMENDED METHOD

REAGENTS—

Dithiol solution—Dissolve 0·5 g. of toluene-3:4-dithiol in 50 ml. of amyl acetate by gentle warming; the temperature should not exceed 40° C., and the solution should be prepared just before use.

"Spekker" acid—Add 150 ml. of sulphuric acid (sp.gr. 1·84) carefully to 600 ml. of water. After mixing, add 150 ml. of phosphoric acid (sp.gr. 1·75), mix, allow to cool and dilute to 1 litre.

Hydrochloric acid (sp.gr. 1·075)—Dilute 350 ml. of hydrochloric acid (sp.gr. 1·18) with water to 1 litre and adjust the specific gravity to 1·075.

Standard molybdenum solution for calibration—Molybdenum content 0·01 mg. per ml.

PROCEDURE—

Weigh out a 4-mg. sample of the steel, dissolve it in 0·5 ml. of Spekker acid diluted with 1 ml. of water, and oxidise with nitric acid. Alternatively, an aliquot containing the equivalent of 4 mg. of the steel and 0·5 ml. of Spekker acid may be taken from a larger bulk of solution from which other samples have been taken for analysis. Evaporate the sample to fuming, take it up in 3 ml. of hydrochloric acid of sp.gr. 1·075 and cool it in a water-bath. Add 3 ml. of 1 per cent. dithiol solution and allow the test to stand for fifteen minutes with occasional shaking. Then wash the mixture into a separating funnel, using a small amount of amyl acetate; shake it, and allow the layers to separate. After removing and rejecting the aqueous layer, wash the amyl acetate layer with 3 ml. of concentrated hydrochloric acid, which removes the slight cloudiness present in it. Make up the solution to 10 ml. with amyl acetate in a graduated flask, mix, and measure its absorption on the absorptiometer, using a micro cell of suitable size, together with Calorex H 503 and Spectrum Red 607 filters. The molybdenum content is found by reference to standard calibration curves, produced by applying the above method to pure iron solutions to which known amounts of molybdenum solution have been added. All the Spekker readings given in this paper have been obtained with a tungsten lamp in the absorptiometer, but sufficient work has been done with a mercury vapour lamp to show that similar results would be obtained, the calibration curve being similar to that obtained with a tungsten lamp.

TYPICAL RESULTS—

With this technique, a number of steels have been analysed for their molybdenum content, with the results shown in the accompanying Table. The figures given for comparison have been obtained by the normal macro thiocyanate method.

TABLE OF COMPARATIVE RESULTS

Type of steel	Molybdenum % Dithiol method	Molybdenum % Thiocyanate method
Plain carbon	0·04	0·04
„ „	0·09	0·09
„ „	0·15	0·15
Low alloy	0·06	0·06
„ „	0·24	0·22
„ „	0·24	0·24
„ „	0·30	0·31
„ „	0·32	0·34
„ „	0·51	0·52
„ „	0·68	0·68
„ „	1·71	1·68
16% Tungsten ..	0·56	0·55
High speed steel ..	4·22	4·19
13% Cr, 1·3% Nb ..	0·01	nil
18 Cr 8 Ni	1·30	1·31
„	2·72	2·73
„	2·40	2·42
„	3·00	2·99

CONCLUSION—

The method as described above will give reasonably accurate results for the molybdenum content of a 4-mg. sample of any steel likely to be examined.

SUMMARY—

A colorimetric method for the determination of molybdenum, in milligram quantities of steel, is described. The method involves the use of toluene-3:4-dithiol in amyl acetate solution, and is virtually specific for molybdenum in steel.

We are indebted to Mr. B. Bagshawe, Assoc.Met., for his advice on this problem, and to Dr. C. Sykes, F.R.S., of the Brown - Firth Research Laboratories, for permission to publish this paper.

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A Method for the Determination of Tungsten in Steel, using Toluene-3:4-Dithiol. The Removal of Molybdenum Interference by Selective Extraction

BY B. BAGSHAWE AND R. J. TRUMAN

THE traditional methods for the determination of tungsten in steel are based on hydrolytic precipitation of tungstic acid and conversion to the oxide. Such methods are reasonably satisfactory for relatively large tungsten percentages, but below about 1 per cent. of tungsten, hydrolysis from steel solutions is very uncertain and usually incomplete, and in our experience this is still true irrespective of whether precipitation aids such as cinchonine, rhodamine B, etc., are used or not. Tungsten in amounts below about 0·2 per cent. occurring as a residual from scrap contamination in non-tungsten steels is sometimes difficult to detect at all by hydrolysis methods and, in any case, the results are often erratic and usually much below the truth.

Attention was first turned to toluene-3:4-dithiol as the basis of an entirely new principle by Hamence[1] in 1940, and later by Miller and Lowe,[2] and Miller.[3,4,5] Miller's work gave a preliminary indication of the possible application of the reagent as the basis of a semi-micro method for the element in steel. More recent work on the subject is that of Vaughan and Whalley.[6] We have since made a prolonged investigation with a view to devising an accurate quantitative means of determination, of which the method given below is the outcome.

It very soon became obvious that the practical application of dithiol reagent to commercial steels was severely limited by the interference of molybdenum. Molybdenum interference is serious because (*a*) the corresponding molybdenum-dithiol reaction is one of high sensitivity, and (*b*) apart from true molybdenum steels, practically all steels are contaminated with this element as a residual impurity. There is, therefore, no useful scope for the principle of the tungsten - dithiol reaction unless account can be taken of the corresponding molybdenum effect.

Miller claims partial suppression of the molybdenum - dithiol reaction by reduction to the tervalent condition with stannous chloride. Our experience confirms her observation that whilst tervalent molybdenum may not react with dithiol there is interference due to subsequent oxidation resulting in the formation of intermediate brown complexes and, later, of the green molybdenum complex itself. The order of this interference is shown in Table I, giving typical results on steels in presence of quite small amounts of molybdenum.

TABLE I

	Steel	Mo % added	W % found
(*a*)	18 Cr 8 Ni, W- and Mo-free	nil	nil
	,, ,,	0·05	0·04
	,, ,,	0·25	0·21
(*b*)	18 Cr 8 Ni, low tungsten ..	nil	0·12
	,, ,,	0·05	0·15
	,, ,,	0·10	0·19
	,, ,,	0·20	0·28
(*c*)	18 Cr 8 Ni, W steel ..	nil	0·78
	,, ,, ..	0·05	0·82
	,, ,, ..	0·10	0·83

The adverse effect of molybdenum, even in residual amounts, is therefore well marked, and it increases, although in irregular manner, with increasing amounts of molybdenum.

We attempted to remove molybdenum together with iron by ethereal extraction of 0·1-g. steel samples as ferric chloride solution containing sufficient phosphoric acid to prevent tungstic acid hydrolysis. It is known that ether extraction of molybdenum approaches 100 per cent. in presence of excess of iron, but our tests showed that the distribution ratio is reduced by the phosphate ion, necessary in this case to fix tungstic acid, with the result that considerable molybdenum is retained in the acid tungsten phase. There was also some loss of tungsten in the ether phase, but conditions that reduce this effect to a minimum, *i.e.*, increased phosphoric acid concentration, similarly induce increased retention of molybdenum in the acid phase, hence no effective ether separation of the two elements was achieved.

A further attempt to apply the ether separation, but after conversion of molybdenum to its oxythiocyanate complex, also failed, and there was additional interference at a later stage from tin salts introduced to reduce molybdenum for the thiocyanate formation.

Later work has been concentrated on an adaptation of a preferential formation and extraction of the molybdenum - dithiol complex itself, which has been reported in a previous paper from these laboratories[7] as the basis for a microchemical method for molybdenum. Our observations are summarised as follows:

(*a*) In hydrochloric acid solutions of approximately 1·06 sp.gr. the molybdenum - dithiol complex alone is formed. If the acid has sp.gr. below about 1·05 the molybdenum complex forms only very slowly, whilst at sp.gr. 1·08 its formation is accelerated but there is also some formation of the corresponding tungsten complex, and this increases with increasing acidity and is quantitative in acid of sp.gr. 1·16.

(*b*) Below 20° C. the formation of the molybdenum complex may be retarded, and if this occurs some molybdenum may escape extraction and interfere later when the tungsten complex is formed. At temperatures exceeding 30° C. there is a gradually increasing tendency for partial simultaneous formation of the tungsten complex even in acid of sp.gr. 1·06 or thereabouts, with resultant loss of tungsten in the discarded molybdenum extract. We now develop the molybdenum complex over a period of fifteen minutes in a bath controlled within the range 20° to 25° C.

(*c*) A prompt and full development of the molybdenum complex is dependent on the use of a freshly prepared solution of the fresh reagent. This is more fully discussed in a later section of this paper.

(*d*) After the amyl acetate extract of the preferentially formed molybdenum complex has been discarded, the residual acid layer containing the tungsten can be concentrated and extracted in hydrochloric acid of sp.gr. 1·16, when further treatment with dithiol reagent on the boiling water bath results in a quantitative formation of the greenish-blue tungsten complex. This reaction is carried out in presence of stannous ion, otherwise high blanks would be obtained due to ferric chloride solubility in the amyl acetate extract of the tungsten complex.

(*e*) The tungsten - dithiol formation is much less sensitive to stale reagent solution than that of the molybdenum complex, probably owing to the influence of stannous ion in reducing oxidation products of the reagent. If the molybdenum separation is imperfect owing to stale reagent, this will be betrayed by the development of brownish tints along with the tungsten complex, which when pure and free from molybdenum should be bluish-green.

From the above observations the method described below has been derived. It is applied to 15-mg. fractions from 0·5 g. steel samples, is suitable for a wide variety of alloy steel types and moreover provides for the first time a ready means of accurately determining tungsten in low ranges, *i.e.*, from 0 to 1 per cent., with which the hydrolytic precipitation methods fail to give quantitative recovery.

METHOD

REAGENTS—

"Spekker" acid—150 ml. of sulphuric acid (sp.gr. 1·84) and 150 ml. of phosphoric acid (sp.gr. 1·75) made up to one litre with distilled water.

Hydrochloric acid (*sp.gr.* 1·06)—Dilute the concentrated acid with water and adjust to sp.gr. 1·06.

Hydroxylamine sulphate solution—10 per cent. solution in distilled water.

Toluene-3:4-*dithiol solution*—Open a sealed 1-g. phial of the reagent and dissolve in 100 ml. of amyl acetate. The solution thus prepared must be used within a few hours of preparation.

Stannous chloride solution—Dissolve 10 g. of stannous chloride in 100 ml. of concentrated hydrochloric acid.

PROCEDURE—

To 0·5 g. of the sample in a 300 ml. flask (Taylor pattern) add 30 ml. of Spekker acid and 10 ml. of concentrated hydrochloric acid. Heat until dissolved, oxidise with concentrated nitric acid and then evaporate until hydrochloric and nitric acids are expelled and the solution fumes. Extract with 100 ml. of water, boil and transfer to a 500-ml. graduated flask. Cool, dilute to the mark with water, and mix.

Pipette a 15-ml. aliquot representing 15 mg. of sample into a 50-ml. flask and evaporate to fuming. Cool, add 5 ml. of hydrochloric acid (sp.gr. 1·06) to the fumed concentrate, warm gently until all salts are in solution and then cool to room temperature. Add 5 drops of the hydroxylamine sulphate solution and 10 ml. of the toluene-3:4-dithiol reagent solution, allow to stand in a bath at 20° to 25° C. for 15 minutes and shake the solution at intervals throughout this period.

Transfer to a 25-ml. stoppered cylindrical tap funnel, rinsing 3 or 4 times with small portions of amyl acetate. Shake and allow the layers to separate. Draw off the lower acid layer containing the tungsten and reserve it in the original 50-ml. flask. Wash the remaining amyl acetate layer twice consecutively with 5-ml. portions of hydrochloric acid (sp.gr. 1·06), separating and withdrawing the acid layer each time as before. Combine the acid wash layers with the original acid layer reserved in the 50-ml. flask. Discard the washed amyl acetate layer, which contains the molybdenum. Evaporate the acid tungsten solution, carefully at first until dissolved amyl acetate is expelled, then add a few drops of concentrated nitric acid and evaporate to fuming. Add a few more drops of nitric acid during fuming to clear up any charring organic matter, rinse the flask with a minimum of water, and re-fume to expel nitric acid and water.

Add 5 ml. of the stannous chloride solution to the fumed liquid and heat on a boiling water-bath for 4 minutes. Add 10 ml. of toluene-3:4-dithiol reagent solution and continue to heat on the boiling water-bath for a further 10 minutes. Shake the solution at intervals throughout this period.

Transfer to a 25-ml. stoppered tap funnel, rinsing 3 times with 2-ml. portions of amyl acetate. Shake, separate, draw off the lower acid layer, and discard it. Add 5 ml. of concentrated hydrochloric acid to the amyl acetate layer in the separator, repeat the extraction and again discard the lower acid layer (see *Note* below).

Draw off the amyl acetate layer, containing the tungsten, into a 50-ml. graduated flask (previously rinsed free from water, first with alcohol and then with amyl acetate), make up exactly to the mark with amyl acetate and mix.

Measure the absorption of this solution in the Spekker absorptiometer, using the mercury vapour lamp, 4 cm. cells and Ilford Spectrum Red No. 608, and Calorex H.503 filters. Refer the readings to a calibration curve prepared from spectrographically pure iron to which suitable increments of standard sodium tungstate solution have been added.

Note—For steels containing appreciable cobalt further repeated washing is necessary at this stage. After the wash with 5 ml. of concentrated hydrochloric acid, give two further washes with hydrochloric acid (sp.gr. 1·06), and a final wash with concentrated acid.

RANGE—

The method as specified, *i.e.*, on 15-mg. aliquots from an initial 0·5-g. sample weight, gives a calibration range of 0 to 1 per cent. tungsten ≡ 100 drum divisions, with the 4-cm. cells. The range may be extended *pro rata* to cover higher tungsten percentages by using 2-, 1-, or 0·5-cm. cells. For the greatest accuracy in the lowest tungsten ranges, the calibration range may be reduced to 100 drum divisions ≡ 0·5 per cent. of tunsten by making the final reading in a volume of 25 ml. The reading accuracy is then of the order of ±0·005 per cent. of tungsten. With the calibration range as specified in the method, the reading accuracy is within 0·01 per cent. of tungsten over the range 0 to 1 per cent., and this fulfils all normal requirements within this range.

RESULTS—

The foregoing method was proved by tests made on austenitic 18 Cr, 8 Ni tungsten bearing steels with and without synthetic additions of molybdenum, and on synthetic preparations obtained from 18 Cr, 8 Ni steels (spectrographically free from tungsten) treated with various known amounts of molybdenum and tungsten solutions. Results are given in Table II.

TABLE II

Steel	Mo added, %	W added, %	W found, %
18 Cr 8 Ni (spectrographically free from W)	nil	nil	nil
	nil	0·50	0·50
	1·0	0·02	0·02
	2·0	0·05	0·05
	2·0	0·20	0·21
	3·0	nil	0·01
	,,	0·10	0·11
	,,	0·50	0·51
18 Cr 8 Ni steel, 0·78% W	nil	—	0·77
	,,	—	0·78
	3·0	—	0·77
	,,	—	0·78
	,,	—	0·77

These results are all accurate to a tolerance of 0·01 per cent. of tungsten, even when abnormally high percentages, *e.g.*, 3 per cent., of molybdenum are introduced.

EFFECT OF OTHER ELEMENTS—

Of the common steelmaking elements, nickel, chromium, manganese, silicon, vanadium, aluminium, titanium, niobium (columbium), and tantalum do not form coloured dithiol complexes under the test conditions and exert no adverse influence even when present in major alloying amounts.

There is a small extraction of iron by the amyl acetate, which imparts a faint yellowish tinge to the ester layer in complete absence of tungsten, and in presence of very small amounts of tungsten modifies the pale bluish-green of the complex to a yellowish-green. It is, however, easy to distinguish between the pale yellow of "blank" iron and the colour introduced by as little as 0·01 per cent. of tungsten, and in any event the reading value of the yellow component is virtually eliminated by the red filters. Thus pure iron spectrographically free from tungsten gives a blank reading of the order of 0·01 Spekker unit, equivalent to about 0·01 per cent. of apparent tungsten. This is compensated by preparing the calibration from a pure iron base with suitable increments of added sodium tungstate rather than from pure sodium tungstate alone.

There is no increase of "blank" absorption from nickel or chromium, both these elements being preferentially soluble in the acid phase and rejected with it. Titanium, silicon, niobium (columbium), and tantalum may give insoluble hydrolysis products on fuming with mixed phosphoric and sulphuric acids, but there is no need to remove them. They do not occlude tungsten which is fixed as soluble phospho-tungstic acid, and any precipitate is virtually eliminated in taking the 15-ml. aliquot from the 500-ml. initial test volume. Any residual amount of these elements soluble in the test aliquot is entirely without influence. The method, therefore, provides for the first time a simple means of determining tungsten in alloy steels containing niobium (columbium), tantalum, and titanium. On a tungsten-free 0·68 per cent. niobium (columbium) steel 0·30 per cent. of tungsten was found after exactly that amount had been added as a standard solution. A sample of heat-resisting alloy (Ni 70, Cr 20, Fe 3, Ti 2·5 per cent.) gave a residual tungsten content of nil by this method and 0·21 per cent. of tungsten after addition of a further 0·20 per cent. as standard solution, whilst British Chemical Standards No. 209 (18/8 steel with 0·59 per cent. of Ti) gave 0·30 per cent. of tungsten after that amount had been added. Copper forms a black precipitate in the ester layer at the molybdenum extraction stage, but there is no interference and there is no corresponding formation at the tungsten extraction stage.

If large amounts of cobalt are present partition occurs, resulting in a partial transfer of the element to the ester layer, which becomes coloured greenish-blue by the cobalt ion. The extent of cobalt interference is indicated by the figures in Table III.

The effect is only important as it applies to cobalt alloy steels; ordinary plain and alloy steels do not contain sufficient residual cobalt to prejudice the tungsten readings. In any event the effect of as much as 10 per cent. of cobalt can be virtually eliminated by repeated acid washing of the ester phase, as described in the *Note* to the Method, *e.g.*, a plain steel treated with the equivalent of 10 per cent. of cobalt gave no measurable reading, and after addition of the equivalent of 0·5 per cent. of tungsten returned a value for this element of 0·52 per cent.

TABLE III

Steel		Cobalt added, %	Apparent tungsten, %
18 Cr 8 Ni (W nil)	..	—	nil
,, ,,	..	2·0	0·06
,, ,,	..	4·0	0·10
,, ,,	..	6·0	0·15
,, ,,	..	8·0	0·21
,, ,,	..	10·0	0·31

OBSERVATIONS ON THE METHOD OF USING DITHIOL REAGENT—

The reagent as normally supplied to us has been obtained in 1-g. sealed glass phials and occasionally in 5 g. sealed phials. It was known that its keeping properties were limited once the phials were broken, and with 5-g. lots we adopted the practice of with drawing 1-g. amounts for the preparation of solution and immediately re-sealing the tubes each time. In spite of this we experienced many inconsistencies of performance in the early stages of the investigation. It was observed that satisfactory molybdenum separations were only achieved with new solutions prepared immediately before use and the molybdenum separation became erratic when the dithiol solutions were a few hours old, and sometimes when absolutely fresh solutions were prepared from dithiol obtained from phials that had previously been opened and re-sealed. The effect was to give an incomplete conversion of molybdenum into its dithiol complex, and hence its incomplete extraction and removal, the residual molybdenum giving the characteristic brown tints at the later tungsten stage and consequent high values for tungsten.

The effect of stale reagent seemed to be confined to the molybdenum formation, as the corresponding tungsten formation was always complete even when the reagent solution was a week old. We attributed this difference in behaviour to the retarding effect of oxidising decomposition products of the reagent upon the formation of the molybdenum complex, the absence of an analogous effect at the tungsten stage being accounted for by the reducing action of stannous chloride. Stannous chloride is not a suitable reducing agent to have present at the molybdenum separation stage, as tin salts hydrolyse on fuming the extracts, with occlusion of tungsten. After trying several alternative reducing agents we obtained satisfactory results with hydroxylamine sulphate, 5 drops of a 10 per cent. aqueous solution, added immediately before the dithiol addition, being sufficient to restore maximum reagent reactivity with molybdenum, under the conditions specified. With greater amounts of hydroxylamine there is again a tendency to incomplete molybdenum extraction, possibly owing to partial reduction of molybdenum itself.

Since introducing hydroxylamine we have not experienced any difficulty in achieving complete extraction of molybdenum, but as an additional safeguard we prefer to use freshly prepared reagent solutions. Surplus reagent solution can conveniently be reserved for the tungsten extraction stage and used over a period of several days.

SUMMARY—

A method is described for the determination of tungsten in steel, using the reagent toluene-3:4-dithiol. Interference from the corresponding molybdenum - dithiol complex is prevented by forming it preferentially in cold dilute acid solution, and removing it by amyl acetate extraction. The residual acid layer is treated for formation of the tungsten - dithiol complex, which develops quantitatively in hot concentrated acid medium. The complex is extracted with amyl acetate and tungsten determined absorptiometrically. There is little or no interference from other elements and the method is applicable to practically all types of alloy steel, being particularly useful for determinations in residual and other low ranges, where the usual gravimetric methods give poor performance.

We are indebted to the directors of Messrs. Thos. Firth and John Brown, Limited, and in particular to Dr. C. Sykes, F.R.S., of the Brown - Firth Research Laboratories, for permission to publish this paper.

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A Chromatographic Adsorption Method for the Estimation of the Provitamin-A Content of Foodstuffs*

By G. B. RAMASARMA, D. N. HAKIM, and S. D. RAO

A NUMBER of methods have been proposed by various authors for estimating the provitamin-A content of plant materials; these have been critically reviewed by Moon,[1] Seaber,[2] Peterson[3] and, very recently, Booth.[4] Essentially all the methods consist of four steps: (1) extraction of the pigments from the material, (2) saponification to remove oils and hydrolyse xanthophyll esters, (3) separation of the provitamins, collectively termed "carotene," from the associated pigments, and (4) estimation of the carotene by colorimetric or spectrophotometric methods of comparison. The present investigation deals mainly with the third step, which until recently consisted in the application of the Willstätter - Stoll phase partition between light petroleum and 90 per cent. methyl alcohol. This procedure is known to have some serious drawbacks; in particular, (*a*) the separation of kryptoxanthin is not sharp, the pigment being found in both the phases, and (*b*) the inactive lycopene is epiphasic and therefore contaminates carotene.

Further, recent investigations in this laboratory[5] have shown that the partition between light petroleum and methyl alcohol is unsatisfactory in that some coloured but biologically inactive degradation products also remain in the epiphasic layer and are incorrectly estimated as carotene. The presence of such carotene-like artefacts has been observed by some earlier workers also: by Kemmerer and Fraps[6] in the excreta of rats and chickens, by Wiseman and his co-workers[7] in market hays and silages, by Quackenbush *et al.*[8] in A.I.V. silage and by Whitnah *et al.*[9] in the faeces of cows. Various treatments have been suggested for the removal of these interfering pigments, *e.g.*, washing the light petroleum solution with diacetone, passing it through calcium carbonate[10] or shaking it with a small amount of specially prepared magnesium carbonate.[11] Seaber[2] proposed a chromatographic method, using Brockmann's alumina as adsorbent and light petroleum containing 3 per cent, of acetone as developer. Moore[12] reported that a column of dicalcium phosphate adsorbs all the xanthophylls and interfering pigments but allows carotene to pass down unadsorbed.

An additional drawback in the phase separation method is that no distinction is made between β-carotene and the other provitamins, which are only half as active as the former. Neither the inactive lycopene nor the "low-grade" provitamins (mostly α-carotene and kryptoxanthin) appear to be of such rare occurrence or in so negligible an amount as was hitherto supposed. This fact seems to have been realised only in the case of yellow corn and yellow maize, as can be seen from the work of Buxton,[13] Fraps and Kemmerer,[14] White *et al.*,[15] and Sadana and Bashur Ahmad,[15a] who have determined both carotene and kryptoxanthin in order to find out the provitamin-A activity. In a critical application of the chemical method for assessing the provitamin-A activity of any plant material,† it is necessary to estimate the individual provitamins and calculate the activity by using the following formula which is based on the definition of the International Unit:

$$\text{Provitamin-A activity in Internat. Units} = \frac{\mu\text{g. of } \beta\text{-carotene}}{0{\cdot}6} + \frac{\mu\text{g. of other provitamins.}}{1{\cdot}2}$$

Chromatographic adsorption, which offers the only means of separating the individual pigments, has been used extensively in qualitative and preparative work, but not for purposes of quantitative estimation, because considerable amounts of the pigments, varying with their adsorptive affinities, may be lost owing to incomplete elution. Thus, if a mixture of pigments is chromatographed to give a sharp separation of the constituents the loss of xanthophylls and other inactive pigments would be immaterial, but the loss of variable amounts (70 to 90 per cent.) of the provitamins would introduce a factor of uncertainty which makes the procedure unsuitable. It is obvious, therefore, that the elimination of the non-carotene pigments and the separation of the individual provitamins for quantitative estimation are not possible in one and the same chromatographic operation. It was thought, however, that a close approximation to the objectives in view might be achieved if the chromatographic adsorption were carried out in two stages; the first to estimate the "total carotene" (including lycopene, if any) and the second to estimate the relative proportions of the individual pigments in this "total carotene." Dicalcium phosphate prepared according to Moore[12] has been found to fulfil the requirements of the first stage, in which xanthophylls and artefacts should be retained completely and the hydrocarbon pigments allowed to pass down unadsorbed. In the second stage, satisfactory resolution of the pigment mixture was obtained by use of Brockmann's alumina or activated magnesia. Throughout this paper the two stages are referred to as the "first chromatography" and the "second chromatography" respectively.

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* This investigation was completed in February, 1943, but owing to unavoidable circumstances it could not be presented for publication earlier. A preliminary note, however, was published in *Current Science*, 1943, **12**, 21.

† It is to be admitted, however, that with certain foodstuffs serious discrepancies have been observed between the chemically determined activity and that obtained by biological assay. Some of these anomalies may be due to imperfections in the chemical methods already referred to, but there are other important factors influencing the biological availability—*viz.*, the extent of digestion and absorption and the presence of varying amounts of anti-oxidants (Hickman *et al.*[16]; Guggenheim[17]). It has been suggested that a relatively high figure should be fixed for the daily requirements if all the vitamin-A activity is to be derived from vegetable sources, for the activity of the provitamins is relatively low at the higher levels of intake, probably owing to inefficient absorption (Guilbert *et al.*[18]; Vitamin-A Sub-Committee of the Accessory Food Factors Committee, Lister Institute and Medical Research Council[19]). Although from a practical point of view a unit of provitamin A is not the same as a unit of vitamin A, the need for further improvements in the chemical and biological methods of assay cannot be denied.

EXPERIMENTAL

PREPARATION AND PURIFICATION OF THE REAGENTS—

Dicalcium phosphate—Dicalcium phosphate was prepared precisely according to Moore.[12]

Alumina—On account of the scarcity of Brockmann's alumina, it was found necessary to activate the adsorbent and use it over and over again. The used adsorbent was repeatedly washed by heating under reflux with light petroleum (b.p. 60° to 75° C.) containing 3 per cent. of alcohol, until the extract was practically colourless. The adhering solvent was removed, finally by drying at 100° C., and the alumina was heated in a nickel dish over a Meker burner (at a temperature ranging between 200° and 250° C.) for a number of hours and stirred frequently with a glass rod meanwhile. There was an initial darkening due to carbonisation of traces of organic matter, but on continued heating the adsorbent became practically white. It was allowed to cool in a desiccator over sulphuric acid and soon after transferred to glass-stoppered bottles.

Light petroleum ("petroleum ether")—As purified light petroleum was not available, ordinary automobile petrol was distilled and the fraction boiling between 60° to 75° C. was collected and purified according to Castille and Henri[20] by treatment with sulphuric acid and alkaline permanganate.

Methyl alcohol—Methyl alcohol was purified by heating under reflux with aluminium turnings (10 g. per litre) and potassium hydroxide (10 g. per litre) and then submitted to fractional distillation. In order to obtain a 92 per cent. v/v methyl alcohol for the phase separation, 90 ml. of purified alcohol were mixed with 10 ml. of water.

Ethyl alcohol free from aldehyde—This was prepared by treating rectified spirit in a manner similar to the methyl alcohol.

CHROMATOGRAPHIC TECHNIQUE—

The procedure was practically the same as that described by Moore,[12] except that smaller adsorption tubes (1·3 × 12 cm.) were employed. The chromatogram was developed under a vacuum of 40 cm. of mercury instead of the 65 cm. recommended by Moore, in order to avoid excessive loss of solvent under tropical conditions.

In order to determine the extent of the loss that may occur during the first chromatography, solutions containing 10 to 60 μg. of β-carotene were passed through identical columns of dicalcium phosphate; estimation of the carotene content of the filtrates showed that the losses of β-carotene rarely exceeded 1 per cent. This is well within the range of errors inherent in visual spectrophotometric determination. The need for care in the preparation of the phosphate has been stressed by Moore.[2,12]

Preliminary experiments showed that both xanthophylls and xanthophyll esters are retained by a column of dicalcium phosphate. Hence, in this method, both the saponification and the phase separation could normally be dispensed with, thus simplifying the procedure considerably. Nevertheless these operations have been retained in the present investigation, in order to demonstrate the existence of non-carotene pigments in the epiphase.

Some of the early trials, in which no saponification was performed, showed that the presence of oil in the extract may adversely affect the chromatographic separation. No difficulty was experienced with rich sources of carotene, *e.g.*, leafy material, the xanthophylls and artefacts from which formed sharply defined and firmly held bands on the dicalcium phosphate column, but the extracts of poor sources, such as spices, cereals, and nuts, gave rise to diffuse overlapping bands, which showed a tendency to come down on continued washing. This difficulty could be easily overcome by preliminary saponification of the light petroleum extract and removal of the soaps. That the adverse effect was due to the presence of oil was confirmed by the following experiment. Aliquots of an oil-free solution containing carotene, lycopene, xanthophylls, and artefacts were mixed with varying amounts of coconut oil and passed over identical columns of dicalcium phosphate. The pigment that flowed down unadsorbed was in each instance estimated as carotene. When the oil contents were 0, 10 and 20 mg., the carotene values were 37, 37·4, and 37·5 μg. respectively. When the oil contents were 50 and 100 mg., higher carotene values, *viz.*, 40·5 and 40·3 μg. respectively, were obtained, showing that some other pigment besides carotene was washed down. When 200 and 500 mg. of oil were used, the bands were irregular and diffuse.

These results indicate that, under the experimental conditions employed, the presence of oil to the extent of about 20 mg. in the extract does not seriously interfere with the adsorption. With larger amounts, however, saponification is necessary. Generally speaking, saponification seems to be essential when the material under test is rich in oil or poor in carotene content.

PROCEDURE—

Extraction of pigments—Preliminary treatment depended upon the nature of the material. Dried materials like cereals and pulses were ground thoroughly in a porcelain edge-runner mortar to pass an 80-mesh sieve and a sample of the powder was taken for analysis. Fresh materials such as fruits and vegetables were cut into small bits and ground with acid-washed sand; this grinding was preferably repeated after the first extraction with alcohol. The quantity of material taken for analysis was largely dependent upon its carotene content; 0·5 to 5 g. of rich sources were sufficient, while 30 to 50 g. were necessary with cereals and pulses; according to the quantities taken, the volumes of alcohol and ether used for extraction were varied.

Typical example of extraction procedure—30 g. of a powdered sample were weighed into a 500-ml. Erlenmeyer flask, 100 ml. of aldehyde-free ethyl alcohol (95 per cent.) were added and the mixture was heated over a water-bath under a reflux condenser for half an hour. The alcoholic extract was decanted, preferably through a wad of cotton wool, into a separating funnel. The residue was extracted similarly with another 100 ml. of alcohol and the extract was added to the first. The residue was further extracted by heating under reflux for 15 minutes with 100 ml. of a mixture of 2 volumes of light petroleum (b.p. 60° to 75° C.) and 1 volume of alcohol. Finally the residue was washed with 50 ml. of a similar petroleum - alcohol mixture. By this time the provitamins were completely extracted. The extracts were combined and sufficient water was added to bring the alcohol concentration to 75 to 80 per cent. The lower, aqueous alcoholic, layer was drawn off and extracted in a separating funnel with three 50 ml. lots of light petroleum (b.p. 60° to 75° C.). Further extraction was found to yield only xanthophylls.

Saponification and phase partition—The combined light petroleum extract was washed once with water and saponified by heating under reflux for 15 minutes with 25 ml. of 10 per cent. alcoholic potash solution. The chlorophyllins, soaps and excess of alcoholic potash were removed by careful washing. The light petroleum extract was then shaken with an equal volume of 92 per cent. methyl alcohol in a separating funnel and the lower, alcoholic, layer, containing xanthophylls, was drawn off. The epiphase was washed repeatedly with smaller portions of 92 per cent. methyl alcohol until the washings were practically colourless. The combined methyl alcoholic solutions were washed with a small volume of light petroleum, which was added to the epiphasic solution. The combined light petroleum solution was washed free from alcohol, dried over anhydrous sodium sulphate and made up to a suitable volume. The pigment concentration was determined by spectrophotometry and the carotene content of the material calculated. This is the value obtainable by the usual methods employing Willstätter - Stoll phase separation.

First chromatography—An aliquot of this solution (or all the solution, if the carotene content was low) was concentrated under reduced pressure in an atmosphere of nitrogen to about 5 to 10 ml. and passed through a column of dicalcium phosphate. The xanthophylls and artefacts were held strongly at the top, while carotene passed down practically unadsorbed. Lycopene (when present) showed little separation from carotene; its presence could, however, be suspected when the top portion of the carotene band, as it approached the lower end of the tube, appeared dark red in colour. It was found convenient to collect the carotene solution in a separate test tube, which could be suspended in the flask just when the carotene reached the lower end of the column and before any pigment was washed down. The solution was made up to a suitable volume and the gross "carotene" content was estimated by spectrophotometric means.

In some experiments it was observed that a distinct, pale yellow band moved down the column soon after the carotene on continued washing of the column; this was found to be kryptoxanthin. Since, under these conditions, the xanthophylls and artefacts do not move down at all, this offers a means of separately estimating kryptoxanthin in any material.

Second chromatography—The "gross carotene" solution (filtrate from the dicalcium phosphate column) was then concentrated to a small volume under reduced pressure and in a current of nitrogen and was passed through a suitably sized column of Brockmann's alumina. The chromatogram was developed with light petroleum, which separated the constituent pigments into sharply defined bands. The bands were carefully scooped out by means of a spatula and separately eluted with light petroleum containing 3 to 5 per cent. of ethyl alcohol. The eluates were made up to suitable volumes and examined in the spectrophotometer for identification and estimation. The total pigment in the eluates did not account for all that was chromatographed. Usually the recovery was about 90 per cent., but since the three common hydrocarbon pigments—α-carotene, β-carotene and lycopene—differ but slightly in their adsorptive affinities, it was assumed that the losses were shared by them equally. The amounts of individual pigments present in the original solution were calculated accordingly.

Spectrophotometry—In order to identify and estimate the different pigment fractions, their solutions in purified light petroleum (b.p. 60° to 75° C.) were examined in a Gaertner Visual Spectrophotometer. The photometer drum readings were taken at 5-mμ. intervals over the range 430 to 550 mμ. and the extinction (E) values were read off from conversion tables (Brode[22]). The pigments were identified by their characteristic absorption maxima and the concentrations calculated on the basis of the extinction coefficients ($E^{1\%}_{1\,cm.}$ = 2440 at 450 mμ., 2020 at 470 mμ., and 2140 at 480 mμ.; solvent light petroleum, b.p. 60° to 75° C.) obtained for a sample of pure β-carotene isolated from Badami mango fruit.[23] The average of the values obtained for the three wavelengths was taken as the correct figure. For the sake of simplicity, all estimations were made in terms of β-carotene, irrespective of the identity of the particular fraction.

Having thus found the amounts of different provitamins present in the material, the total provitamin-A content expressed in International Units was calculated according to the formula given on p. 194.

Results for a number of food materials found by this procedure are shown in the accompanying Table. For comparison, values given in the *Health Bulletin No.* 23, Third Edition (1941), published by the Government of India, have also been included in the table; most of them were determined by De,[24] by means of a phase separation method.

Discussion

Our results clearly show that plant materials that have been dried and stored for some time contain appreciable amounts of epiphasic artefacts. These are wrongly estimated as carotene by the usual phase partition methods. By chromatography on a column of dicalcium phosphate the impurities can be removed without loss of carotene.

Sekhon[25] found that loss of carotene on dehydration of carrots was small. Analysis of a sample of dehydrated carrots (supplied by Messrs. Parry & Co.) indicated that over 20 per cent. of the epiphasic pigment consisted of destruction products showing no definite absorption spectrum. Our work shows that more reliable results will be obtained if the chromatographic method is employed for such dehydrated foods.

Another interesting fact brought out is the multiplicity of epiphasic pigments. It is generally assumed that most of the carotene of plant materials consists of β-carotene and that lycopene and kryptoxanthin are of rare occurrence. Among the materials tested, more than half the "apparent carotene" of coriander seeds and nearly 90 per cent. of that of mace seems to be lycopene. The carotene of chillies and carrots contains nearly 25 per cent. of the α-isomer. Again, the provitamin A occurring in papaya fruit is almost entirely kryptoxanthin. The few results presented here clearly indicate the need for similar detailed analyses of the common Indian foodstuffs.

Owing to the exigencies of the war, there has been a considerable increase in the consumption of dehydrated foods, while processed and canned foods promise to come into a more extensive use than ever before. Various investigators have studied the carotene losses when foodstuffs are subjected to different cooking processes. It is highly probable that in all this work the observed provitamin-A values were vitiated by interference from non-carotene pigments. A re-investigation employing improved chromatographic methods seems to be desirable.

PROVITAMIN-A CONTENT PER 100 G. OF MATERIAL

			Determined by the authors			
Material	Botanical name	*Health Bulletin* No. 23 µg.(=I.U.) I	Phase partition µg. II	First chromatography µg. III	Second chromatography (calculated) µg. IV	Vitamin-A activity (calculated) I.U. V
Bengal gram	*Cicer arietinum*	316	224	162	$b = 162$	270
Green gram	*Phaseolus radiatus*	158	109	26	$b = 24$; $? = 2$	42
Red gram	*Cajanus indicus*	220	125	100	$b = 100$	166
Horse gram	*Dolichos biflorus*	119	74	17	$b = 17$	28
Lentil	*Lens esculenta*	450	70	25	*	42
Whole wheat	*Triticum vulgare*	108	21	6	*	10
Cholam	*Sorghum vulgare*	136	20	13	*	22
Fenugreek seeds ..	*Trigonella foenugraecum*	160	660	260	$b = 240$; $? = 20$	420
Mace	*Myristica fragrans*	—	3580	2150	$L = 1976$; $b = 174$	290
Red chillies (dry) ..	*Capsicum annuum*	576	11200	6230	$b = 4420$ $a = 1620$; $? = 190$	8870
Pistachio nut	*Pistacia vera*	240	176	140	$b = 126$; $a = 14$	220
Gingelly seeds ..	*Sesamum indicum*	100	21	12	—	20
Mustard	*Brassica juncea*	270	178	149	$b = 141$; $? = 8$	242
Coriander seeds ..	*Coriandrum sativum*	1570	230	100	$L(?) = 55$; $b = 45$	73
Mango fruit (Badami)	*Mangifera indica*	4800	—	5000	$b = 5000$	8330
Papaya fruit	*Carica papaya*	2020	—	1280	$c = 1150$; $b = 130$	1175
Carrots (dehydrated)	*Daucus carota*	—	37800	29800	$b = 22350$ $a = 7450$	43460

a = α-carotene; b = β-carotene; L = lycopene; c = kryptoxanthin; ? = doubtful identity;
* = not analysed, assumed to be all β-carotene.

Note:—Column I: Values reported in the *Health Bulletin No.* 23.
Column II: Values obtained by the authors, employing a similar phase-separation method.
The differences between I and II are mostly due to differences inherent in the samples analysed.
Column III: Values obtained by subjecting II to chromatography over dicalcium phosphate.
(II)–(III): Non-carotene pigments wrongly estimated as carotene by the phase-separation method.
Column IV: Composition of III in terms of individual pigments (calculated from the results obtained by subjecting III to chromatography over Brockmann's alumina).
Column V: Activity in International Units calculated from IV.

Finally, a word of caution is necessary with regard to the first chromatography. There are many variables in the experimental procedure—the adsorptive power and particle size of the dicalcium phosphate, the size of the column and the manner of packing, the vacuum applied during chromatography, and so forth; unless due care is taken, considerable errors may be introduced. At the outset, all these experimental conditions should be standardised to give quantitative recoveries of carotene and the details strictly adhered to subsequently. It is further recommended that every new lot of adsorbent should be tested for its proper performance before it is brought into use.

In the second chromatography (over Brockmann's alumina) which seeks to estimate the relative proportions of the individual pigments, it is not quite correct to suppose that the losses due to incomplete elution are shared equally by the constituents. By analysis of "synthetic" mixtures, it has been found, as might be expected, that larger amounts of the more strongly adsorbed pigment are lost. The consequent errors are not high, but improvements in this direction are desirable.

Subsequent to the completion of this work (February, 1943), several groups of workers[26–30] have proposed chromatographic methods for the estimation of the true carotene content of processed foods, feeding stuffs, and the like. It should be mentioned, however, that few of these methods, with the exception of those of Kemmerer and co-workers,[31,32] go beyond the estimation of the total carotene content. The large number of investigations of a similar nature that have been reported during recent years shows the extent to which the subject is deservedly attracting the attention of workers interested in nutrition.

SUMMARY

An improved method for the estimation of the provitamin-A content of foodstuffs has been described. It involves application of chromatographic adsorption in two stages, using suitable adsorbents: the first to estimate the total "carotene" and the second to ascertain the relative proportions of the constituent pigments. This permits the estimation of the individual provitamins and therefore a more precise estimate of the total provitamin-A activity of the material under test.

A few food materials have been analysed by this improved method and the results are presented. The results indicate that large errors might be introduced in the analysis of dried and stored materials, owing to the presence of certain modified carotenoid pigments which are devoid of any provitamin-A activity, but are estimated as carotene by the usual methods employing the Willstätter - Stoll phase separation. These impurities can be removed by chromatographic adsorption over dicalcium phosphate, the carotene recoveries being quantitative. The possible formation of epiphasic non-carotene pigments during the course of storage, cooking, canning or dehydration of foodstuffs has been discussed and the need for a thorough re-investigation by improved chromatographic methods has been stressed. In order to eliminate errors due to the presence of lycopene and the "low grade" provitamins, the second chromatography should be carried out and the individual pigments estimated.

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DEPARTMENT OF BIOCHEMISTRY
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The Determination of Carotene in Dried Grass

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(Read at the Meeting of the Society on April 2, 1947)

A NEW method for the determination of carotene in dried grass is being put forward by a Carotene Committee,* which was originally formed through the Crop Driers Association, and in 1941 proposed a tentative method of analysis.[1] This was called Method B, two other methods, A and C, having been under consideration at that time.

In order to point out the defects of this old method and show the progress that has since been made, it is necessary to give an outline of the tentative method as published in 1941.

METHOD B OF 1941—

The sample of dried grass is ground to a fine powder with sand and extracted with a 3:1 mixture of light petroleum of b.p. 40° to 60° C. and acetone, in a Soxhlet or drip-type extractor. The extract is shaken up with a concentrated solution of potash in methyl alcohol to convert the chlorophyll present into potassium chlorophyllin, which is removed with water. The remaining extract now contains carotenoids and xanthophyll, and the xanthophyll is removed by means of a 9:1 mixture of methyl alcohol and water. The amount of carotene in the light petroleum solution thus obtained is then estimated colorimetrically.

From the work of Seaber,[2] Kon and Thompson,[3] Moore[4] and Mann,[5] it was demonstrated by chromatography that there is in grass a petrol-soluble carotenoid that does not exhibit the properties of β-carotene and has no biological activity. It was believed that this particular "carotene" forms on the average 30 per cent. of the total carotenoids in grasses and therefore analysis of a grass meal by Method B gives an untrue picture of its biological worth.

Furthermore, it was shown that it is not always possible to remove all the chlorophyll as potassium chlorophyllin, and a trace of chlorophyll in the final solution makes the colour estimation fallacious.

Any method which would give the true β-carotene content of a grass meal would, therefore, have to embody the following three important features:

(1) It must be capable of separating β-carotene completely from the non-active carotenoids of grass.
(2) It must yield for the final colorimetric estimation a solution entirely free from any forms of chlorophyll.
(3) It must be a method in which there is no likelihood of isomerisation taking place.

Points (1) and (2) have been mentioned above, and for (3) it is necessary to consider the work of Zechmeister.[6] He showed that β-carotene was capable of spontaneous isomerisation in solution and gave molecular extinction curves of the all-*trans* compound and mixtures of its stereoisomers (see Fig. 1).

Table I gives the $E^{1\%}_{1\,cm.}$ values of β-carotene over the wavelength range 451 to 447 mμ., calculated from the above molecular extinction curves.

TABLE I

Wavelength mμ.		$E^{1\%}_{1\,cm.}$
451·0	all *trans*	2575
450·5		2505
450·0		2435
449·5		2365
449·0		2295
448·5		2225
448·0		2155
447·5		2085
447·0	much isomerisation	2015

When a solution is being analysed colorimetrically for β-carotene the $E^{1\%}_{1\,cm.}$ value is usually taken to be 2500 at 450 mμ. From the figures in Table I it is obvious that, if isomerisation has taken place and the $E^{1\%}_{1\,cm.}$ at 450 mμ. is assumed to be 2500, inaccuracies will be incurred in determining the β-carotene present.

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* Members of the Carotene Committee—Mr. R. O. Davies (*Chairman*), Dr. V. H. Booth, Dr. A. Green, Mr. J. Greenbaum, Mr. A. W. Hartley, Mr. T. Barton Mann, Dr. F. E. Moon, Dr. W. A. G. Nelson, Mr. W. M. Seaber, Mr. H. H. Ward, Dr. H. Wilkinson, Mr. R. F. Wright.

Zechmeister has postulated the possibility of the existence of twenty isomers of β-carotene within the range from all *trans* to all *cis* (see Fig. 2) and he has been able to identify several of them.

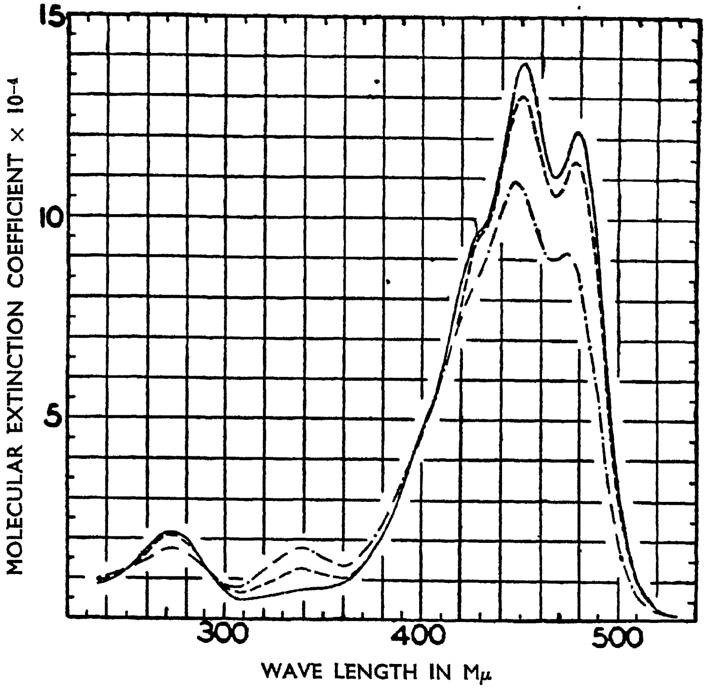

FIG. 1. Molecular extinction curves of β-carotene in hexane: ——, fresh solution of the all-*trans* compound; — — —, mixture of stereoisomers after heating under reflux in darkness for 45 min.; — · —, mixture of stereoisomers after iodine catalysis at room temperature in light. (From *J. Amer. Chem. Soc.*, 1943, **65**, 1523.

Bearing in mind the facts mentioned above, Mann, Seaber, Green and Hartley (who are members of the present Carotene Committee) collaborated with a view to finding an improved method for the determination of carotene in grasses. As a result of their investigations a chromatographic method called Method D was suggested.

METHOD D—

One g. of the sample is ground to a fine powder with sand and extracted with a 3:1 mixture of light petroleum of b.p. 40° to 60° C. and acetone, as in Method B. The mixed solvents are removed almost completely by evaporation on the water bath and the last traces are blown off with a stream of carbon dioxide to minimise oxidation. The dry residue is dissolved in light petroleum (b.p. 40° to 60° C.) and then passed through a column of bone meal (Mann's findings on the use of bone meal as an adsorbent have already been recorded[5]). The filtrate is then examined for β-carotene colorimetrically in the usual way.

The salient points emerging from the analysis of grass meal by Method D were:

(1) The filtrate from the bone meal column contained carotene which had the full biological activity of β-carotene, whilst the "carotene" fraction retained by the bone meal had no biological activity.
(2) The filtrate contained no chlorophyll.
(3) The absorption spectrum of the carotene in the filtrate coincided with that of all-*trans*-β-carotene.

It was now clear that a method had been found which gave a much more accurate determination of β-carotene in grass than the previous ones. By the combined efforts of the whole Committee, it was shown that Method D gave more reproducible results than those obtained by Method B.

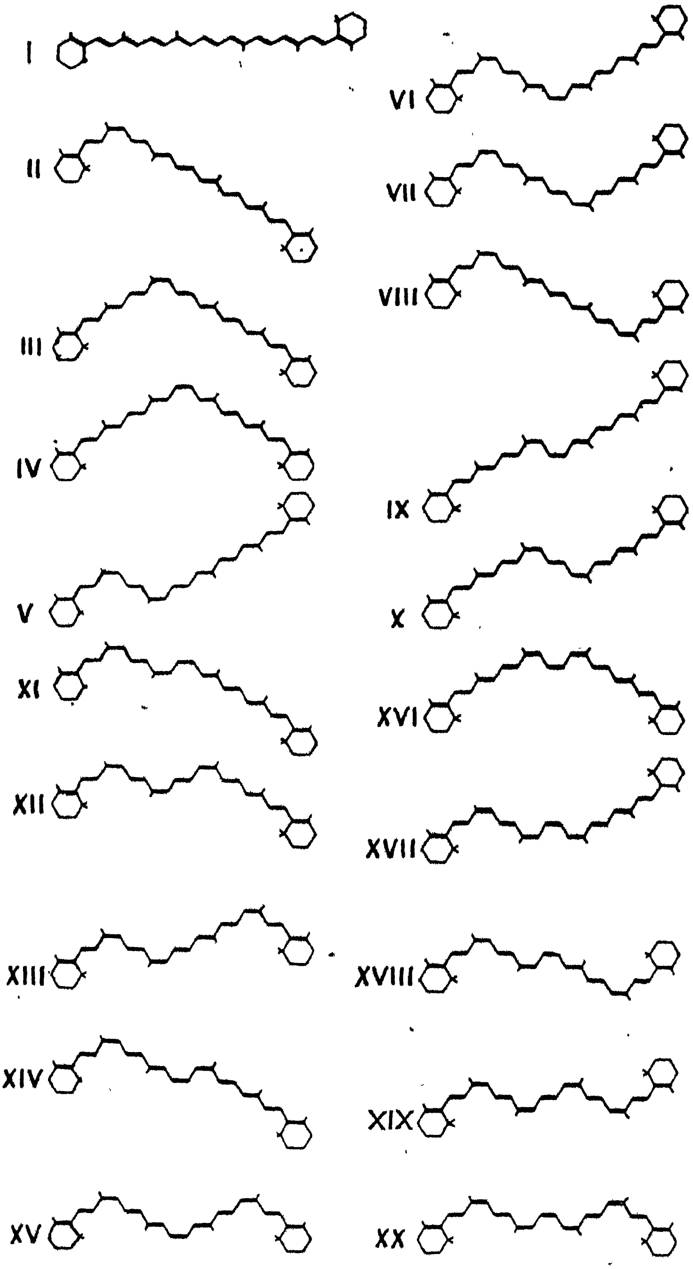

Fig. 2. Skeleton models of the twenty possible stereoisomers of β-carotene: all-*trans*-β-carotene, three mono-*cis*-β-carotenes, six di-*cis*-β-carotenes, six tri-*cis*-β carotenes, three tetra-*cis*-β-carotenes, and all-*cis*-β-carotene.

After this stage had been reached, an improvement in the method of extraction was brought about through the work of the Lever Bros. team consisting of Wilkinson, Edisbury and Gridgeman. The process of grinding with sand, followed by extraction with a 3:1 mixture of light petroleum of b.p. 40° to 60° C. and acetone, was replaced by extraction with light petroleum of b.p. 80° to 100° C. alone, without any initial grinding. This is now adopted in the method finally recommended by the Committee—Method E—which is described below.

METHOD E—NOW RECOMMENDED

Boil from 1 to 2 g. of grass meal with 50 to 60 ml. of light petroleum of b.p. 80° to 100° C. under reflux for 1 hour on a steam bath. Cool the flask and contents and filter the extract through sintered glass or any other suitable type of filter, or merely decant directly, on to a 2-inches by 1-inch column of bone meal. Rinse the flask and residue with small quantities of light petroleum (light petroleum of b.p. 40° to 60° C. may be used at this stage, for ease of subsequent removal if this happens to be desirable). Apply suction to the bone meal column, elute with light petroleum of b.p. 40° to 60° C. and concentrate if necessary. Estimate the carotene in the eluate, colorimetrically.

NOTES—

Boiling can be done in large Kjeldahl flasks, which have been found to provide sufficient condensing effect.

The bone meal should be extracted before use with a 3:1:1 mixture of petrol, acetone, and ether.

The bone meal should be of particle size passing a 120-line sieve but retained by a 200-line sieve. (Arrangements are being made for the preparation of a standard bone meal which can be supplied to anyone interested in this type of work.)

The bone meal column can be used for a large number of determinations. After repeated use it may be washed with acetone, which removes the bulk of the pigments. A further wash with light petroleum prepares the column for further tests.

The whole Committee carried out from 60 to 70 analyses on a sample of dried grass by Method D and an equal number by Method E. The average results by these two methods for all practical purposes can be considered to be identical. For this particular sample the average figures were:

By Method D: 335 mg. per kilo. By Method E: 330 mg. per kilo.

Further comparative average figures for a large number of tests were as follows:

By Method D: 129 mg. per kilo. By Method E: 135 mg. per kilo.
" " " 275 " " " " " " 279 " " "

It has been shown that light petroleum of b.p. 80° to 100° C., without preliminary grinding of the sample with sand, is as efficient for the extraction of carotene as any other solvent or mixture of solvents. Its efficiency is only equalled by that of soaking the sample in the dark and in the cold for 48 hours with light petroleum of b.p. 40° to 60° C. This latter method, of course, is undesirable if an analysis is required quickly, as is usually the case. Previously, attempts to extract with boiling light petroleum of b.p. 40° to 60° C. had been made, but had been found to be inefficient. It seems that the light petroleum of b.p. 80° to 100° C. is much superior because of the higher temperature attained during extraction.

As mentioned previously, it was believed that about 30 per cent. of the so-called "carotene" obtained by Method B was not β-carotene. As a matter of academic interest as well as commercial importance, the Committee set out to get a more definite figure. For this purpose, 42 samples were analysed by Methods B and E, and it was found that the results by Method E were only 69 per cent. as great as those by Method B. Again this is an average figure, some meals giving, say, 60 per cent. and others 80 per cent., depending on the age of the meal and certain other factors.

From this work the following conclusions can be drawn:

(1) Light petroleum of b.p. 80° to 100° C. is an efficient solvent for the extraction of the carotenoids of grass.
(2) Bone meal is an efficient adsorbent for the separation of β-carotene from the other pigments in grass.
(3) Method E can be carried out more rapidly and easily than any other method.

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3. Kon, S. K., and Thompson, S. Y., *J. Agric. Sci.*, 1940, **30**, Pt. IV, 636.
4. Moore, L. A., *Ind. Eng. Chem., Anal. Ed.*, 1940, **12**, 726.
5. Mann, T. Barton, ANALYST, 1944, **69**, 34.
6. Zechmeister, L., *Chem. Revs.*, 1944, **34**, No. 2, 267.

DISCUSSION

Mr. W. M. Seaber said that in the past the estimation of carotene had been lengthy and tedious. He had been connected with this problem for a long time and had been interested to see the method develop in the direction of simplicity. He had tried the new method, not only on dried grass but also in general work, and had found it convenient and rapid. They owed a great deal to Mr. Barton Mann for bringing to light the advantages of bone meal as an adsorbent of non-carotene substances, and to the chemists at Messrs. Lever Brothers for introducing the use of light petroleum of higher boiling-point range than that previously used for the extraction. He was pleased to have taken part in the work of the Committee.

Mr. N. T. Gridgeman said that he had recently, on behalf of the Carotene Committee, statistically analysed the results of the final collaborative experiment in which methods D and E were compared. It had emerged that the reproducibility of the two methods was substantially the same (coefficient of variation $= 4$), and that method E yielded slightly, yet significantly, higher results (by about 3 per cent.) than method D. As in so many analytical techniques, reproducibility was found to be better within laboratories than between laboratories, and there may be good reason to believe that inter-laboratory differences (coefficient of variation $= 8$) were largely due to non-uniformity of instruments. Many laboratories perforce used "abridged" spectrophotometers, *e.g.*, photoelectric absorptiometers dependent upon optical filters and absorption standards. In β-carotene estimation potassium dichromate solution enjoyed an undeserved popularity as a suitable calibration medium—perhaps by false analogy with its undoubted virtues as a visual standard. The steepness of the slope of the absorption curve of potassium dichromate in the region of 450 mμ. rendered it highly unserviceable, and the position was worsened by the fact that most types of nominally identical optical filters exhibit marked variations in their transmission characteristics. He hoped that eventually a wholly satisfactory standard would be found; meantime perhaps the best expedient was to use petrol solutions of pure β-carotene.

Mr. K. A. Williams said he would like to support in the strongest possible terms Mr. Gridgeman's strictures on the use of potassium dichromate as a yellow colour standard. Whilst its use in visual colorimetry was defensible because of the limits of sensitivity of the human eye, it could not be defended for more accurate work. In spectroscopy or photo-electric measurements any standard must have an absorption curve very closely approximating to that of the test colour; it was but rarely that the curve of potassium dichromate did this.

Mr. T. Barton Mann said that from the point of view of spectroscopy one would naturally take readings at the peak of absorption. In photo-electric instruments employing filters he had found potassium dichromate relatively insensitive in so far as variations in concentration near that recommended, *i.e.*, 0·0158 per cent. ($\equiv$ 1 μg. of carotene per ml.) did not appreciably affect the graph. There was little difference between 0·0158 and 0·0164 per cent. solutions. He agreed that search should be made for an alternative standard, preferably a dye the colour of which would not be affected by change of *p*H.

Dr. C. H. Lea asked if differences in exposure to light in the course of analysis might cause a variable amount of change of the carotene.

Dr. Nelson pointed out that in method E there was no evaporation of solvent from the extract, as there was in method D.

Mr. Barton Mann said that oxidised carotene would be adsorbed on bone meal and not pass into the solution to be measured. The use of the higher-boiling light petroleum reduced risk of oxidation, because the higher temperature enabled the carotene to be extracted without previous grinding of the dried grass with sand. It would seem that the higher temperature removed the moisture of the grass meal and permitted the solvent to penetrate more readily.

Mr. Seaber remarked that light petroleum of b.p. 80° to 100° C. was apt to be rather variable in character and composition.

Mr. Williams suggested that perhaps *n*-heptane might prove more satisfactory. It had a boiling-point range of 98° to 100° C., and to ensure freedom from aromatic constituents it could be washed with sulphuric acid.

Mr. Barton Mann said that on the use of various petroleum fractions of lower or higher boiling-point ranges he would particularly like to draw the attention of analysts to a point that had arisen in the collaborative work of the Committee, *viz.*, that with change of solvent from one of lower to one of higher boiling-point there was a concomitant shift of peak absorption from a lower to a higher wavelength. For example, with light petroleum of b.p. 40° to 60° C. the E max. was 447 to 448 mμ., but with that of b.p. 80° to 100° C. it was 452 to 454 mμ. Dr. J. R. Edisbury had very kindly checked these findings and had furnished the speaker with the refractive indices of two such petroleum fractions, *viz.*, one of b.p. 40° to 60° C., 1·3656 and one of b.p. 80° to 100° C., 1·4002. These differences in absorption and refractive index were significant, perhaps not so much for users of spectrophotometers, by whom the extinction would normally be obtained at peak absorption, but certainly with colorimeters and instruments employing filters, where a change of wavelength would automatically introduce a change of colour. Pending further work along these lines it would seem advisable to adhere to the estimation "carotene in light petroleum of b.p. 80° to 100° C."

There could be no question that the method proposed did produce a solution containing the true carotene of grass meals and that intra-laboratory concordant results could be obtained. The question of inter-laboratory concordance appeared to be entirely a matter of instrument calibration, and it was to be hoped that the Committee would tackle this problem, possibly along the lines of a master reference instrument of the photo-electric spectrophotometer type to which the owners of colorimeters could refer.

Dr. E. C. Wood asked if the accuracy of the new method had been checked by "recovery" experiments with known added amounts of pure β-carotene, and also if the method could be adapted to the analysis of substances containing vitamin A as well as carotenoids. He had read recently of an organic dyestuff, forming a stable solution, with an absorption spectrum sufficiently similar to that of carotene to make it a satisfactory standard instead of potassium dichromate. Perhaps one of the investigators could comment on this.

Mr. Barton Mann said he had no knowledge of any work on the recovery of carotene added to grass meals. The difficulty of such work would be very great. He had determined the efficacy of recovery of β-carotene from fat, using the bone meal treatment. A freshly prepared 0·03 per cent. standard, after saponification and extraction with ether, was subjected to the whole process of bone meal chromatography, and the figure returned was 0·0283 per cent. Having regard to the severity of the manipulation and the inherent errors of visual spectrophotometer estimation, one could conclude that the loss by such chromatography was nil or negligible.

With regard to vitamin A, Dr. T. W. Goodwin had found from his work with cod liver oil that vitamin A ester passes bone meal and vitamin A alcohol is adsorbed by it. If Dr. Wood had in mind the application of the method to biological material, for which saponification is usually necessary, then carotene would pass into the filtrate and vitamin A, as the alcohol after saponification, would remain adsorbed. He would refer Dr. Wood to "The separation of vitamin A from xanthophyll" (Mann, Analyst, 1943, **68**, 233); "A Chromatographic Method for Separating Free and Esterified Vitamin A" (Glover, Goodwin, and Morton, *Biochem. J.*, 1947, **41**, 94); "Relationship between Blood Vitamin A Level and Liver Stores in Rats" (*Ibid.*, 97).

Mr. Gridgeman said that the yellow substance referred to by Dr. Wood was methyl orange in aqueous solution, which had been suggested as a standard for β-carotene by R. J. Taylor in a recent paper (Analyst, 1946, **71**, 566). But it applied to β-carotene only in chloroform solution, in which the peak absorption occurred at about 460 mμ. No "control" experiments of the type Dr. Wood had in mind had been carried out, nor did he think they would be very valuable, as the separation problem was the extraction of carotene from grass cells.

In reply to a question by Mr. Bacharach, as to whether statistical analysis had isolated any inter-personal variations within laboratories, the speaker said that the inter-personal error had been studied in one laboratory and found to be negligible; but with colorimetric (in contrast with absorptiometric) methods some observational disagreements would almost certainly have to be reckoned with.

Later in the discussion Mr. Gridgeman deprecated the use of nitrogen for the removal of final traces of solvent, on the ground that it often contained some oxygen. Carbon dioxide was objectionable because of frosting of the cylinder valves. Hydrogen was always used in his laboratory and seemed to be the most suitable of the common gases for the purpose.

Notes

THE DETERMINATION OF LEAD AS IODATE IN GLASS ANALYSIS

We have recently carried out determinations of the lead content of three samples of lead glass (SiO_2 56%, PbO 30%, Al_2O_3 1%, CaO 0·5%, K_2O 7·5%, Na_2O 5%) by the gravimetric iodate method described by C. H. R. Gentry and L. G. Sherrington.[1] In every instance the lead iodate precipitate obtained showed a yellow discoloration, and a subsequent spectroscopic examination of one precipitate showed the presence of iron and titanium.

The results obtained are shown in Table I, together with results obtained in parallel determinations in which the lead was determined by separating and weighing it as sulphate.

Table I

Sample	PbO as iodate Per cent.	PbO as sulphate Per cent.	Difference Per cent.
A	29·66; 29·53	28·98; 28·94	0·6; 0·59
B	30·07; 29·98	29·48; 29·44	0·59; 0·54
C	30·42; 30·18	29·87; 29·75	0·55; 0·43

With sample A, the lead iodate precipitates, after being weighed, were dissolved through the sintered glass crucibles with warm dilute caustic soda solution, 20 ml. of hydrochloric acid were added and the solutions evaporated to small volume to eliminate iodic acid. Ten ml. of sulphuric acid were then added to each beaker and the contents evaporated to strong fuming. The determination of the lead by weighing as lead sulphate was completed in the usual manner. By this method the PbO content was found to be 29·07 and 28·94 per cent., compared with 28·98 and 28·94 per cent. found by the direct determination as sulphate.

The yellow coloration of the original lead iodate precipitates, together with the spectrographic evidence, suggested that iron might be present as an impurity. To explore further the influence of small amounts of iron on the determination of lead as iodate a series of standards was prepared containing known added quantities of iron. The iron was added in the form of ferric nitrate to a solution of pure lead nitrate of which the PbO content was approximately that of a 1-g. sample of the glasses under examination. The lead content was determined gravimetrically as iodate, as before. In Table II are given the results, together with the lead content determined as lead sulphate. Assuming that the iron is completely co-precipitated with the lead as ferric iodate, the presence of 0·07 per cent. of iron (expressed as Fe_2O_3) in a glass should theoretically give rise to a positive error of 0·20 per cent. ($Fe(IO_3)_3 \times 0{\cdot}1374 = Fe_2O_3$; $Pb(IO_3)_2 \times 0{\cdot}4005 = PbO$.) The actual difference found was 0·25 per cent.

TABLE II

PbO as sulphate Per cent.	Fe_2O_3 added Per cent.	PbO as iodate Per cent.	Difference Per cent.	
29·80	0·0	29·91	0·11	
				0·26
	0·07	30·17	0·37	
				0·25
	0·14	30·42	0·62	
				0·50
	0·28	30·92	1·12	

The iodate method has also been tried on glasses containing both barium and strontium in addition to lead; the results are shown in Table III.

TABLE III

Sample	PbO as iodate Per cent.	PbO after conversion of iodate to sulphate Per cent.
A	19·4; 19·4	18·5; 18·5
B	21·2; 21·2	19·6; 19·7

These precipitates also were badly discoloured and obviously contained iron. However, the iodic acid precipitation is a valuable method for effecting a preliminary separation of lead from barium and strontium.

Gentry and Sherrington point out that ferric iron precipitates with the lead but state that "the traces of iron in lead glasses are too insignificant to interfere with the lead determination."

Our results indicate that the iodate method for the determination of lead in glass can be applied only to samples known not to contain more than very small amounts of iron; 0·07 per cent. of Fe_2O_3, for example, can give an increase of 0·25 in the percentage figure for PbO.

If the lead iodate precipitate shows a yellow discoloration it is advisable to redissolve it and to reprecipitate the lead as sulphate.

REFERENCE

1. Gentry, C. H. R., and Sherrington, L. G., ANALYST, 1946, **71**, 31.

P. M. C. PROFFITT
R. C. CHIRNSIDE
January, 1947

RESEARCH LABORATORIES,
THE GENERAL ELECTRIC CO.
WEMBLEY

NOTE ON THE PREPARATION OF *CYCLO*HEXANE FOR SPECTROSCOPIC WORK

IN a number of analytical processes involving the use of absorption spectroscopy *cyclo*hexane is commonly employed as a solvent. Its suitability for this purpose depends on the absence of absorption bands, and for this reason traces of benzene, from which *cyclo*hexane is prepared by hydrogenation, must be removed.

By the method of Dolin[1] it was found that a recent delivery of *cyclo*hexane contained 0·4 per cent. of benzene.

Traces of benzene are usually removed by sulphonation, a process that involves repeated shaking with oleum followed by successive washes with water, permanganate solution and water. The *cyclo*hexane is then dried over calcium chloride and redistilled.

This process of purification is time- and labour-consuming, and it has been found that the benzene can be completely removed by adsorption on passing the *cyclo*hexane through a column of silica gel. This method has the merit of simplicity, it requires no attention, and the operation is conveniently allowed to run overnight.

The apparatus consists of a glass tube approximately 1100 mm. long, with an internal diameter of 10 mm. To one end is fused the cone portion of a B14 standard joint, and a sintered glass disc is sealed into the tube at a position approximately 20 mm. from the other end. A reservoir is made from a glass tube, 300 mm. long and 45 mm. wide, tapered at the bottom and fitted with the socket portion of a B14 standard joint.

The tube is packed with powdered silica gel, of a particle size which passes a 30-mesh sieve but is retained on a 150-mesh sieve. The packing is done by small additions, after each of which the tube is gently shaken by tapping on a pad of filter papers. Approximately 100 g. of silica gel are required to pack the tube, which is then fixed in a vertical position. The cylindrical glass reservoir is attached to the top of the tube and charged with 350 to 400 ml. of *cyclo*hexane. The liquid percolates slowly through the column, the *cyclo*hexane going forward and the benzene remaining behind in accordance with their relative adsorbabilities. At the end of 2½ hours, benzene-free *cyclo*hexane may be collected from the bottom of the column at the rate of 16 ml. per hour. If the operation is continued overnight the rate of collection very slowly drops to 14 ml. per hour by the following morning.

The purified *cyclo*hexane collected during the night is tested for freedom from benzene by the formolite reaction, which consists in adding 1 drop of the sample to a mixture of 10 ml. of concentrated sulphuric acid containing 0·5 ml. of 40 per cent. formaldehyde solution. In presence of traces of benzene a yellow to red coloration at once appears. Thereafter the *cyclo*hexane coming from the column is collected in 20-ml. portions, each being tested for freedom from benzene by the formolite reaction. The first fraction to show traces of benzene indicates the "break point" of the column and the end of the run.

Using the conditions and apparatus described above, a test run was started at 12 noon, and by 4 p.m. the following day 320 ml. of benzene-free *cyclo*hexane had been collected. The result of a spectroscopic test confirmed the absence of benzene and hence the suitability of the sample. In this run the "break point" occurred after 350 ml. of *cyclo*hexane had been collected.

Silica gel is an inexpensive adsorbent and a fresh 100-g. charge has been used for each run. There would, however, appear to be no difficulty in reconditioning the silica for further use.

I wish to thank the Government Chemist for permission to publish this note.

REFERENCE

1. Dolin, *Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 242.

S. A. ASHMORE
February, 1946

DEPARTMENT OF THE GOVERNMENT CHEMIST
THE GOVERNMENT LABORATORY
LONDON, W.C.2

Ministry of Food

STATUTORY RULES AND ORDERS*

1947 No. 231. The Flour Confectionery (Control and Maximum Prices) (Amendment) Order, 1947. Dated February 10, 1947. Price 1d.

This amending Order, as from February 10, 1947,

(1) *expressly excludes black puddings and white (or mealy) puddings from the definition of flour confectionery;*

(2) *provides a new method of ascertaining the maximum price of flour confectionery where some or all of the ingredients are supplied by the purchaser;*

(3) *increases the ceiling price of flour confectionery from* 1*s.* 6*d. to* 2*s.* 6*d. a lb.* (*where the combined fat and sugar content amounts to* 14 *per cent. or more and the combined fat, sugar and dry egg solids content amounts to* 40 *per cent. or more*), *while at the same time reducing the ratio of maximum price to ingredient cost where* 1*s.* 6*d. per lb. is exceeded;*

(4) *increases the maximum price of uncooked pastry from* 9*d. to* 1*s.* 0*d. a lb.*

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* Obtainable from H.M. Stationery Office. Italics signify changed wording.

1947 No. 650. The Food Standards (Mustard) (No. 2) (Amendment) Order, 1947. Dated April 11, 1947. Price 1d.

As from April 16, 1947, *the standard for* mustard, compound mustard and mustard condiment *is hereby reduced from* 0·35 *per cent. to* 0·28 *per cent. of allyl isothiocyanate.*

— **No. 701 The Edible Oils and Fats (Control of Sales) (Amendment No. 2) Order, 1947. Dated April 19, 1947.** Price 1d.

From April 23, 1947, *this amending Order removes the restrictions on the sale of olive oil, almond oil, peach kernel oil (or apricot oil), teaseed oil and hazelnut oil to trade users.*

It revokes the previous amending Order, S.R. & O. No. 386 *of* 1946, *and substitutes the following definition of Edible Oil for that given in Article* 1 *of the principal Order, S.R. & O. No.* 672 *of* 1944.

"Edible oil" means any oil or fat used or capable of being used for human food, or in the manufacture, preparation or treatment of human food, and includes any oil or fat used or capable of being used for the greasing of tins, trays, or other utensils used in the cooking or preparation of food for human consumption, but does not include—

(*a*) "butter," "cooking fats" or "margarine" (as defined in the Fats, Cheese and Tea (Rationing) (No. 2) Order, 1946), or suet or peanut butter or peanut paste;
(*b*) any aromatic oil used for flavouring purposes;
(*c*) petroleum or any product of petroleum, as defined in the Petroleum (No. 2) Order, 1942;
(*d*) olive oil, almond oil, peach kernel oil (or apricot oil), teaseed oil, and hazelnut oil, including any mixture of any two or more of those oils but not including any mixture of any one or more of those oils with any other oil.

— **No. 757. The Labelling of Food (Amendment) Order, 1947. Dated April 25, 1947.** Price 1d.

From May 1st, 1947, *this amending Order exempts the following foods from the labelling requirements of the Labelling of Food Order,* 1946, *by adding them to Table B of the First Schedule of the order,* which table specifies foods wholly exempt from Article 2 of the Order when prepacked for sale as such and partly exempt when forming an ingredient of some other food—

Fresh fruit and vegetables other than potatoes (not including fruit or vegetables which are bottled, frozen, dried or otherwise processed, but so that for this purpose cleaning or the removal of extraneous or inedible matter shall not be regarded as processing).

Single toffee apples.

British Standards Institution

A FEW copies of the following draft Specifications, issued for comment only, are available to interested members of the Society and may be obtained on application to the Secretary, J. H. Lane, 7–8, Idol Lane, London, E.C.3.

Draft Specifications prepared by Technical Committee FCC/4—Solvents.

CH(FCC)9402—Draft Revised B.S.573 Dibutyl Phthalate.
CH(FCC)9401—Draft Revised B.S.508 Normal Butyl Alcohol.
CH(FCC)9403—Draft Revised B.S.574 Diethyl Phthalate.
CH(FCC)9404—Draft Revised B.S.575 Carbon Tetrachloride.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Biochemical

Colorimetric Estimation of Proteins in Various Body Fluids. A. A. Albanese, V. Irby, and B. Saur (*J. Biol. Chem.*, 1946, **166**, 231–237)—It was found that when alkaline solutions of proteins, isolated from body fluids by treatment with trichloroacetic acid or sodium sulphate solutions of appropriate concentration, were submitted to the modified Sakaguchi reaction of Albanese and Frankston (*Ibid.*, 1945, **159**, 185) arginine values were obtained which, on multiplying by a suitable factor, resulted in protein values closely approximating to those obtained by the micro-Kjeldahl method.

Method—Total proteins of blood—Transfer 0·5 ml. of plasma to 10 ml. of 10 per cent. trichloroacetic acid in a 15-ml. conical centrifuge tube, leave for 10 min. at room temperature, and centrifuge for 10 min. at 3,000 r.p.m. Decant the supernatant liquid into a 100-ml. Kjeldahl flask and treat in the usual manner for the estimation of non-protein nitrogen by the micro-Kjeldahl method. Wash the precipitated protein by re-suspension in 5 ml. of 5 per cent. trichloroacetic acid, centrifuge, and decant the supernatant liquid. Dissolve the washed protein in 10 ml. of 10 per cent. sodium hydroxide solution and use 1-ml. aliquots for the arginine estimation.

Globulin in blood—Add 0·5 ml. of plasma to 10 ml. of 22 per cent. sodium sulphate solution in a round-bottomed centrifuge tube and incubate at 37° C. for 3 hours or more. At the end of the incubation period, add 3 ml. of ether, shake vigorously, and centrifuge for 15 min. at 3,000 r.p.m. Separate the protein that collects at the ether-water interface from both liquid layers by careful decantation, and wash the protein cake by re-suspension in 10 ml. of 22 per cent. sodium sulphate and 3 ml. of ether, centrifuge, and decant as described before. Dissolve the washed protein in 10 ml. of 10 per cent. sodium hydroxide solution, and warm the mixture to remove residual ether. Use 1-ml. aliquots for the arginine estimation. When only limited amounts of the sample are available, the estimations can be performed on 0·1 to 0·2 ml. of plasma by proportionately reducing the volume of the reagents.

Total proteins in urine and ascitic fluid—To 5 ml. in a 15-ml. centrifuge tube add 2 ml. of 30 per cent. trichloroacetic acid, leave for 10 min., and centrifuge for 10 min. at 3,000 r.p.m. Discard the supernatant liquid and re-suspend the protein in 5 ml. of 5 per cent. trichloroacetic acid and again centrifuge. Dissolve the protein in 5 ml. of 10 per cent. sodium hydroxide solution and use 1-ml. aliquots for the arginine estimation.

Globulin in urine and ascitic fluid—Incubate 10 ml. for 3 hours or more at 37° C. with 10 ml. of 44 per cent. sodium sulphate solution in a 50-ml., round-bottomed centrifuge tube. Add 3 ml. of ether, shake, centrifuge, and re-suspend the protein in 10 ml. of 22 per cent. sodium sulphate solution and 3 ml. of ether. Discard the supernatant liquid and dissolve the globulin in 10 ml. of 10 per cent. sodium hydroxide solution.

Colour development—To the 1-ml. aliquots, add 5 ml. of water and 1 ml. of a 0·1 per cent. solution of α-naphthol in 95 per cent. ethanol. After 5 min., add 1 ml. of 0·06 *N* sodium hypochlorite and, exactly 1 min. later, 2 ml. of 20 per cent. urea solution. Leave for 5 min., and evaluate the colour in a Klett-Summerson colorimeter with filter S.54. Treat a 1-ml. aliquot of a standard containing 100 µg. of arginine per ml. and a reagent blank in the same way, and calculate the amount of arginine in the sample from the value found for the standard. Multiply the arginine values by 19·2, a factor derived from the observed mean arginine content (5·2 per cent.) of albumin and globulin of the body fluids. The albumin content is the difference between the total protein and the globulin values. F. A. R.

Colorimetric Estimation of Tocopherol. A. Emmerie (*Rec. Trav. Chim.*, **1946, 65,** 489–492)—The effect of cholesterol on the reaction between *dl*-α-tocopherol and the ferric chloride-dipyridyl reagent was investigated, as cholesterol had been alleged by other workers to interfere with the reaction. This was confirmed with a commercial sample of cholesterol, and it was shown that inhibition was much stronger in glacial acetic acid than in alcohol solution. Purified cholesterol, however, did not inhibit the reaction and the substance responsible for the inhibition could be removed from the commercial material by filtration through Floridine XS. The nature of this impurity is not known, but ergosterol was without effect. F. A. R.

Microbiological Estimation of Amino Acids. III. Methionine. C. M. Lyman, O. Moseley, B. Butler, S. Wood, and F. Hale (*J. Biol. Chem.*, **1946, 166,** 161–171)—The method uses *Leuconostoc mesenteroides* as the test organism and a medium in which most of the amino-acid nitrogen is supplied by hydrogen-peroxide-treated peptone. The results were compared with those obtained when *Streptococcus faecalis* R was used.

Method—Maintain the organism *L. mesenteroides* P–60 by weekly transfers as stabs in solid medium containing peptonised milk 1 per cent., tryptone 1 per cent., filtered tomato juice 200 ml. per litre of medium, and agar 1 per cent. Inoculate with washed cells from 18 hours-old cultures grown on a liquid medium of the same composition except for the omission of the agar.

The composition of the basal medium for the estimation of methionine by means of this organism is given in Table I.

TABLE I

MEDIUM* FOR DETERMINATION OF METHIONINE WITH *Leuconostoc mesenteroides*

H_2O_2-treated peptone	15	g.
Glucose	40	,,
Sodium acetate	24	,,
Ammonium chloride	12	,,
l(—)-Tryptophan	100	mg.
dl-Tyrosine	200	,,
l(—)-Cystine	200	,,
Adenine sulfate	20	,,
Guanine	20	,,
Uracil	20	,,
Aneurine	2	,,
Pyridoxine	4	,,
Calcium pantothenate	4	,,
Riboflavine	4	,,
Nicotinic acid	4	,,
Biotin	10	µg.
Folic acid (synthetic)	3	,,
p-Aminobenzoic acid	0·2	,,
Salt solution 1†	10	ml.
,, ,, 2‡	10	,,
,, ,, 3§	10	,,

Neutralise and dilute to 1 litre.

* Medium for 200 cultures of 10 ml. final volume (5 ml. of the above medium per culture).

† Salt solution 1, K_2HPO_4 25 g., KH_2PO_4 25 g., water 250 ml.

‡ Salt solution 2, $MgSO_4.7H_2O$ 10·0 g., NaCl 0·5 g., $MnSO_4.4H_2O$ 0·5 g., water 250 ml.

§ Salt solution 3, $FeSO_4.7H_2O$ 0·5 g., water 250 ml.

To prepare the hydrogen-peroxide-treated peptone, dissolve 50 g. of Bacto-peptone in 250 ml. of water and add 250 ml. of 2 *N* hydrochloric acid after the peptone is completely dissolved. Add 2·8 g. of 30 per cent. hydrogen peroxide, leave overnight at room temperature, and then steam for 30 min. at atmospheric pressure. Stir while hot, cool, neutralise with sodium hydroxide, and steam for one hour to destroy the excess of hydrogen peroxide. Dilute to a final volume of 1 litre.

The acid produced after 4 days' incubation is a measure of the growth of the organisms, the general procedure for carrying out the assays being the same as that used in assays with *Lactobacillus arabinosus*, except that a constant-temperature water-bath at 35° C. was used instead of an incubator, and the period of incubation was 4 days instead of 3 days.

The medium used for the estimation of methionine by means of *S. faecalis* R is given in Table II.

TABLE II

MEDIUM* FOR DETERMINATION OF METHIONINE WITH *Streptococcus faecalis* R

Glucose	40 g.
Succinic acid	20 ,,
Sodium acetate (anhydrous)	6 ,,
Adenine sulphate	10 mg.
Guanine	10 ,,
Uracil	10 ,,
Xanthine	10 ,,
Riboflavine	1 ,,
Nicotinic acid	2 ,,
Pyridoxamine	0·8 ,,
Aneurine chloride	0·4 ,,
Calcium pantothenate	0·4 ,,
Biotin	2 µg.
p-Aminobenzoic acid	2 ,,
Folic acid (synthetic)	10 ,,
Salt solution 1	10 ml.
,, ,, 2	10 ,,
,, ,, 3	10 ,,
dl-Alanine	400 mg.
l(+)-Arginine	400 ,,
dl-Aspartic acid	800 ,,
l(—)-Cystine	400 ,,
dl-Glutamic acid	800
Glycine	400
l(—)-Histidine	400
dl-*Iso*leucine	400
dl-Leucine	400
l(+)-Lysine	400
dl-Phenylalanine	400
l(—)-Proline	400
dl-Serine	400
dl-Threonine	400
l(—)-Tryptophan	200
dl-Tyrosine	400
dl-Valine	400

Add 12 gm. of sodium hydroxide pellets and finish neutralising with sodium hydroxide solution. Dilute to 1 litre.

* Medium for 200 cultures of 10 ml. final volume (5 ml. of the above medium per culture).

The assay procedure is the same as with *L. mesenteroides* except that the period of autoclaving the tubes for sterilisation is 10 min. and the incubation time is 3 days.

The results obtained by the two microbiological methods were in very close agreement with one another and with the results obtained by the method of McCarthy and Sullivan (*Ibid.*, 1941, **141**, 871) as modified by Csonka and Denton (*Ibid.*, 1946, **163**, 329). F. A. R.

Iodimetric Estimation of Glucose in Urine and Blood. E. C. Noyons (*Rec. Trav. Chim.*, 1946, **65**, 485–488)—*Method*—Prepare a protein-free filtrate of blood by the Fujita-Iwatake method, using cadmium sulphate and sodium hydroxide; the Folin-Wu method gives unsatisfactory results, apparently because it fails to remove certain interfering substances that are precipitated by means of the cadmium reagent. Add to this filtrate 20 ml. of 0·1 *N* iodine and 5 ml. of a buffer solution prepared by dissolving 3·6 g. of KH_2PO_4 in water, adding 37·5 ml. of 4 *N* sodium hydroxide, and diluting to 100 ml. After 30 min., acidify with 10 ml. of 25 per cent. hydrochloric acid and back-titrate with 0·1 *N* sodium thiosulphate. In the estimation of glucose in diabetic urine, it is advisable to treat the urine with Norit carbon (0·5 g. per 100 ml.) before addition of the iodine. The results obtained by this method agreed with those obtained by other methods, and recoveries of added glucose were satisfactory. F. A. R.

Colorimetric Estimation of Methionine in Proteins and Foods. M. J. Horn, D. B. Jones, and A. E. Blum (*J. Biol. Chem.*, 1946, **166**, 313–320)—Difficulties were encountered in applying the method of McCarthy and Sullivan (*Ibid.*, 1941, **141**, 871) as modified by Hess and Sullivan (*Ibid.*, 1943, **151**, 635) to the estimation of methionine in protein foods; a procedure was devised that overcame these difficulties. The results were in close agreement with those obtained by a microbiological method (*cf.* following abstract).

Procedure—Hydrolyse the protein either by means of acid or by digestion with papain. For the acid hydrolysis, heat 1 g. of the protein under reflux for 18 hours with 25 ml. of 20 per cent. hydrochloric acid. Concentrate the hydrolysate to about 5 ml., heat with a small quantity of Norit and filter. Wash the residue on the filter with hot water and dilute the combined filtrate and washings to 100 ml. with water. Alternatively, carry out a papain digestion as described by Horn and Jones (*Ibid.*, 1945, **157**, 153; *Abst.* ANALYST, 1945, **70**, 266) but, before the digest is made up to volume, pass nitrogen through the solution for 15 min. to remove hydrogen cyanide.

Pipette duplicate, 2-ml. samples of the hydrolysate into Evelyn colorimeter tubes and to each add 3 ml. of water and 1 ml. of 5 *N* sodium hydroxide. To one of the tubes add 0·1 ml. of 10 per cent. sodium nitroprusside solution. Leave for 10 min. with frequent shaking and then add 2 ml. of 3 per cent. glycine solution to each tube. Leave for another 10 min. with frequent shaking and then add slowly to each tube, with constant agitation, 2 ml. of concentrated orthophosphoric acid. After 5 mins., evaluate the colour in an Evelyn colorimeter using a No. 540 filter. Use a tube containing 10 ml. of water to adjust the instrument to 100. Calculate the methionine content from the reading so obtained by reference to a calibration curve prepared as follows:

Prepare a standard methionine solution by dissolving 100 mg. of *dl*-methionine in 5 ml. of 20 per cent. hydrochloric acid, and dilute to 100 ml. with water. Into an Evelyn colorimeter tube put 2 ml. of a solution of the following composition (mg. per 100 ml.):

Alanine ..	20	Leucine ..	100
Arginine ..	48	Lysine ..	60
Aspartic acid	60	Norleucine ..	30
Cystine ..	*10	Phenylalanine	40
Glycine ..	5	Proline ..	80
Glutamic acid	210	Serine ..	60
Histidine ..	30	Threonine ..	45
Hydroxyproline	10	Tryptophan	12
*Iso*leucine ..	50	Tyrosine ..	65
		Valine ..	60

* A relatively high proportion of cystine was added, since casein contains less of this amino acid than most proteins.

together with 3 ml. of water, 1 ml. of 5 *N* sodium hydroxide and 0·1 ml. of 10 per cent. sodium nitroprusside solution. Into other colorimeter tubes put 2 ml. of the amino-acid solution, 1 ml. of the standard methionine solution containing amounts ranging from 0·1 to 1·0 mg., 2 ml. of water, 1 ml. of 5 *N* sodium hydroxide, and 0·1 ml. of 10 per cent. sodium nitroprusside solution. After 10 min., add 2 ml. of 3 per cent. glycine solution to each tube and continue the procedure as described for the protein hydrolysate. The logarithms of the readings plotted against concentrations give a straight line. Recoveries of methionine added to an acid hydrolysate of arachin ranged from 96 to 102 per cent. of the theoretical. F. A. R.

Microbiological Estimation of Methionine in Proteins and Foods. M. J. Horn, D. B. Jones, and A. E. Blum (*J. Biol. Chem.*, 1946, **166**, 321–326)—*Lactobacillus arabinosus* 17–5 was used, together with the following basal medium:

Glucose	20	g.
Sodium acetate (anhydrous) ..	12	„
Salts A		
K_2HPO_4	1	„
KH_2PO_4	1	„
Salts B		
$MgSO_4.7H_2O$	400	mg.
$MnSO_4.4H_2O$	20	„
NaCl	20	
$FeSO_4.7H_2O$	20	
Adenine	100	
Guanine	100	
Uracil	100	
Aneurine chloride	2·0	mg.
Pyridoxine hydrochloride ..	0·4	„
Calcium pantothenate ..	0·4	„
Riboflavine	0·4	„
Nicotinic acid	0·8	„
p-Aminobenzoic acid	0·4	„
Biotin	0·01	„
Folic acid	30	μg.*
dl-Alanine	80	mg.
l(+)-Arginine	96	„
dl-Aspartic acid	240	„
dl-Glutamic acid + H_2O ..	940	„
Glycine	400	„
l(−)-Histidine hydrochloride + H_2O	54	„
l(−)-Hydroxyproline	20	„
dl-*Iso*leucine	50	„
dl-Leucine	400	„
dl-Lysine hydrochloride ..	300	„
dl-Norleucine	120	„
dl-Phenylalanine	160	„
l(−)-Proline	140	„
dl-Serine	240	„
dl-Threonine	180	„
l(−)-Tryptophan	200	„
l(−)-Cystine	400	„
l(−)-Tyrosine	130	„
dl-Valine	240	„

Solution brought to 1000 ml. vol. *p*H 6·8

* "Potency 5000."

Procedure—Carry the organism on stab cultures, using a medium of the following composition: glucose 1 g., Bacto yeast extract 1 g., and agar 1·5 g. per 100 ml. Store in a refrigerator and subculture each month. Prepare the inoculum for the assay by transferring a small amount of growth from the stab culture to a centrifuge tube containing 10 ml. of a medium consisting of glucose 1 g., Bacto yeast extract 1 g., and "Difco liver infusion" 100 ml. Incubate at 35° C. for 24 hours, centrifuge, wash the cells with sterile saline, and suspend in 5 ml. of sterile saline. Use one drop of the suspension to inoculate each tube in the assay.

Hydrolyse a sample containing about 1 g. of protein by heating for 24 hours under reflux with 25 ml. of 20 per cent. hydrochloric acid. Concentrate the hydrolysate to a small volume and boil for a few minutes with a little Norit. Filter and dilute the filtrate to 100 ml. with water. Take 10-ml. aliquots of this solution for assay, adjust to *p*H 6·8, and dilute to 1 litre. Store the solutions under toluene in a refrigerator.

Put 5-ml. quantities of the basal medium into a series of tubes and to one set add sufficient of a standard solution of *l*(−)-methionine to give concentrations ranging from 0 to 20 μg. per 10 ml.; prepare each concentration in quadruplicate. To another set of tubes add 1, 2, 3, and 4 ml. of the protein hydrolysate. Dilute all the solutions to 10 ml., autoclave at 15 lbs. for 15 min., and inoculate with one drop of the *L. arabinosus* suspension. Incubate at 35 to 36° C. for 72 hours, and titrate the lactic acid with 0·1 *N* sodium hydroxide, using bromothymol blue as indicator. Calculate the methionine content of the protein hydrolysate by reference to the standard curve. Satisfactory agreement was obtained in replicate experiments, and recoveries of methionine added to casein or gelatin hydrolysates ranged from 96 to 102 per cent.

F. A. R.

Organic

2:4-Dinitrophenylsemicarbazide. Reagent for Aldehydes and Ketones. J. L. McVeigh and J. D. Rose (*J. Chem. Soc.*, 1945, 713–714)—2:4-Dinitrophenylsemicarbazide (Kniphorst, *Rec. Trav. Chim.*, 1925, **44**, 724) gives with aldehydes and ketones characteristic derivatives which are insoluble in water and very sparingly soluble in the common organic solvents.

Preparation of 2:4-*dinitrophenylsemicarbazide.* To 150 g. of 50 per cent. w/w aqueous solution of hydrazine hydrate in 2 litres of boiling ethanol, add 200 g. of N-2:4-dinitrophenyl-N′-nitrourea (Reudler, *Ibid.*, 1914, **33**, 35) during 0·75 hr. Each addition causes vigorous effervescence (nitrous oxide) and, after 30 min., crystals of the semicarbazide begin to separate. Heat under refluxing conditions on the steam-bath for 1 hour, cool to 10° C., and collect the yellowish-brown crystals, wash with a little alcohol, and dry (yield, 143 g.). Recrystallise from alcohol; m.p. 195–196° C. (decomp.). The hydrochloride, from alcohol, has m.p. 202–203° C. (Found: N, 25·1. $C_7H_7O_6N_5.HCl$ requires N, 25·2 per cent.)

Method of test, using benzaldehyde. To 0·31 g. of 2:4-dinitrophenylsemicarbazide in 40 ml. of boiling alcohol, add 0·15 g. of benzaldehyde and 1 drop of concentrated hydrochloric acid. Boil for 5 min., during which time yellow crystals begin to separate, and cool to 10° C. Collect the yellow needles (0·31 g.), wash with 5 ml. of alcohol, and dry; m.p. 232° C. (decomp.), unchanged by recrystallisation from chlorobenzene. (Found: N, 21·0. Calc. for $C_{14}H_{11}O_6N_5$:N, 21·3 per cent.). The 2:4-dinitrophenylsemicarbazones of the following aldehydes and ketones are given below, melting points of the derivatives being given in brackets.

Formaldehyde (207° decomp.), acetaldehyde (225° decomp.), crotonaldehyde (230° decomp.), *n*-butyraldehyde (196° decomp.), *iso*butyraldehyde (209°), furfuraldehyde (227°), benzaldehyde (232°[*]), *p*-dimethylaminobenzaldehyde (247° decomp.), anisaldehyde (248°), *o*-chlorobenzaldehyde (248°), cinnamaldehyde (231° decomp.), methyl ethyl ketone (235° decomp.), methyl *n*-propyl ketone (207° decomp.), methyl *iso*butyl ketone (225° decomp.), methyl *n*-hexyl ketone (192° decomp.), ethyl acetoacetate (179°), 5-diethylaminopentan-2-one (175°), di-*iso*propyl ketone (202–203°), *cyclo*hexanone (234°), benzoin (228°), chalkone (226°), benzylideneacetone (246°), acetophenone (245° decomp.), deoxybenzoin (228°).

Avoid ethers as solvents for recrystallising the semicarbazones; from dioxan, many of the substances retain solvent of crystallisation which is removed completely only on prolonged heating at 110° C. under reduced pressure.

The semicarbazones can be hydrolysed by boiling with 2 *N* sulphuric acid, and the aldehyde or ketone recovered by steam distillation.

E. M. P.

Identification of Aryl Iodides. J. C. Nichol and R. B. Sandin (*J. Amer. Chem. Soc.*, 1945, **67**, 1307–1308)—Iodoso chlorides have been used for the identification of some aryl iodides. The derivatives are quickly prepared in good yield and require no purification. They can be titrated quantitatively with standard sodium thiosulphate solution after addition of an acid and potassium iodide (Sandin, *Chem. Revs.*, 1943, **32**, 257, 259). They have the disadvantages that the melting-points or decomposition points depend upon the rate of heating, and that iodoso chlorides are not stable over a period of time. However, the authors have obtained consistent "melting-points" by heating rapidly at a uniform rate, and carrying out the determinations shortly after the preparation of the derivative. The melting-point of an iodoso chloride

BENZENE DERIVATIVES

Dichloride of benzene derivative substituted as below	M.p., ° C., uncorr.		Equivalent weight		
	After 10–15 mins.	After 1 hr.	After 15 min.	After 1 hr.	Calc.
I	120–121	118–119	138	138·2	137·5
1–CH_3,2–I	91	90–91	146·5	145·9	144·5
1–CH_3,3–I[a]	104–105	99–100	145·7	145·4	144·5
1–CH_3,4–I[a]	100	96–100	146·8	153·1	144·5
1–Cl,2–I[b]	96–97	96–97	155·5	155·5	154·7
1–Cl,3–I[b]	99–100	97–98	155·3	157·3	154·7
1–Cl,4–I	115	115	156·2	156·7	154·7
1–Br,3–I	108–109	108–109	181·4	185·1	177·0
1–Br,4–I	124–125	123–124	178·9	181·3	177·0
1:4–di–I[c]	147–148	146–147	203·8	201·3	200·5
1–C_6H_5,4–I	110	108	178·8	187	175·5
1:4–di–CH_3,2–I	87–89	84	153·3	153·9	151·5

[a] An equal-part mixture melts at 80–82° C. [b] An equal-part mixture melts at 80° C.
[c] An iodoso chloride is produced instead of the expected di-iodoso chloride.

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[*] Kniphorst (*loc. cit.*) gives 223° C.

is taken as the temperature at which the tube begins to fill with gas and liquid. All melting-points were determined with Shriner and Fuson's apparatus (*"The Systematic Identification of Organic Compounds,"* 2nd Edn., John Wiley & Sons, Inc., New York, 1940, pp. 85–87).

Preparation of iodoso chlorides—Dissolve 1 g. of solid aryl iodide, or 1 ml. of liquid compound, in 3 to 15 ml. of dry chloroform, depending on the solubility. Cool to 0° C., and pass a slow current of dry chlorine through the solution. Usually the iodoso chloride separates pure as yellow crystals. If the crystals do not separate, add 10 ml. of low-boiling ligroin. Filter by suction, wash with ligroin, and air-dry for 10 min., maintaining the suction.

Determination of equivalent weights—Place 0·5 to 1 g. of the freshly prepared and air-dried iodoso chloride in a 250-ml. Erlenmeyer flask with 50 ml. of water, 10 ml. of chloroform, 10 ml. of glacial acetic acid, and a small excess of solid potassium iodide. Titrate the liberated iodine with standard sodium thiosulphate solution.

Results are shown in the Table opposite.

Unsatisfactory results were obtained with *o*-bromoiodobenzene and 4-iodo-1:3-dimethylbenzene.

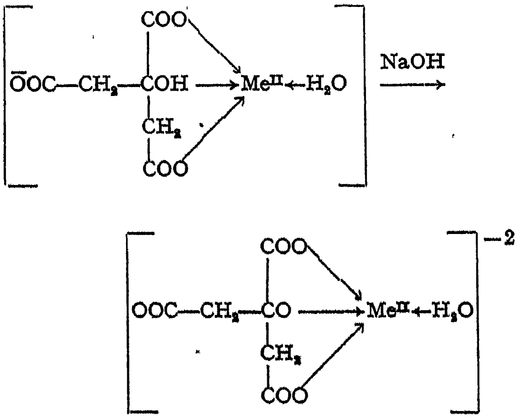
E. M. P.

Determination of "Active" Hydrogen. E. A. Braude and E. S. Stern (*J. Chem. Soc.*, 1946, 404–406)—The use of the reaction with magnesium methyl iodide for the determination of "active" hydrogen by measurement of the methane evolved was first suggested by Tschugaeff (*Ber.*, 1902, **35**, 3912), and further developed by Hibbert and Sudborough (*J. Chem. Soc.*, 1904, **85**, 933) and by Zerewitinoff (*Ber.*, 1907, **40**, 2023; ANALYST, 1907, **32**, 299) who, owing to the high vapour pressure of diethyl ether, used di-*iso*amyl ether as the solvent. Other solvents such as anisole and pyridine have been tried, but di-*iso*amyl ether continues to be employed (*cf.* Hollyday and Cottle, *Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 774). The preparation of the di-*iso*amyl ether reagent is laborious owing to the difficulty of rendering the solvent completely anhydrous and free from *iso*amyl alcohol; it is relatively expensive, and is not stable for long periods unless special precautions are taken. Moreover, the apparatus is cumbersome, and difficult to clean and dry. A method has now been developed, with diethyl ether as solvent, in which these disadvantages are overcome and which requires no special apparatus other than a simple nitrometer. It is applicable, however, only to relatively non-volatile compounds with functional groups reacting completely at room temperature.

Procedure—To prepare the reagent, treat 35 g. (0·25 g.-mol.) of methyl iodide with 8 g. of magnesium in 200 ml. of sodium-dry ether and decant the liquid into a 300-ml. brown-glass, stoppered bottle in which air has been displaced by oxygen-free nitrogen. After some hours, re-decant the reagent and adjust its volume to 250 ml. The apparatus consists of an ordinary 50-ml. nitrometer filled with dry mercury, a rubber stopper carrying a small, cylindrical, graduated, and stoppered dropping funnel being fitted into the cup. Introduce about 5 ml. of dry ether above the mercury in the measuring limb of the nitrometer. Weight 50 to 200 mg. of the sample in a wide specimen tube, place this in the cup of the nitrometer and insert the stopper and dropping funnel tightly. Adjust the mercury to the zero mark with the tap of the nitrometer and that of the graduated funnel open, and then close the latter, and test the nitrometer for absence of leaks. Place 3 ml. of the reagent in the funnel and allow about 2 ml. to enter the specimen tube in the cup, drop by drop, until no further reaction occurs. Adjust the mercury level in the reservoir limb to slightly above that in the measuring limb and allow the apparatus to stand for 3 to 5 min. Finally adjust the mercury level and measure the volume of gas evolved. If the tap of the dropping funnel is greased, a blank determination must be made, and this should not exceed 2 ml. per ml. of reagent used. If draughts are excluded and room temperature is not too high, constant readings can be obtained over periods of at least 15 min. No difficulties arising from slow absorption of oxygen by the reagent have been experienced. From the nitrometer reading deduct the volume of reagent run in and the blank value (if any) to obtain the volume of methane evolved at a pressure equal to atmospheric pressure (p) less the vapour pressure (p′) of ether at the temperature of the determination. If W is the weight in grams of the sample, of molecular weight M, and T is the absolute temperature, the theoretical volume (ml.) of methane per atomic equivalent of "active" hydrogen is given by $\frac{22400\,\mathrm{pT}}{273(\mathrm{p}-\mathrm{p}')} \times \frac{\mathrm{W}}{\mathrm{M}}$. Determinations with six standard substances, *viz.*, *cyclo*hexanol, benzyl alcohol, phenol, quinol, acetic acid, and benzoic acid gave results consistent to within ±1 or 2 parts in 100.

A. O. J.

Metallic Complexes of Tartrates and Citrates; their Structure and Behaviour in Dilute Solutions. I. Cupric and Nickelous Complexes. M. Bobtelsky and J. Jordan (*J. Amer. Chem. Soc.*, 1945, 67, 1824–1831)—Measurements of conductivity, light absorption, and *p*H, and polarograph experiments, show that several bivalent cations, in dilute solution, form complexes with trisodium citrate and disodium tartrate,, but not with free citric or tartaric acid. In this paper a study of the copper and nickel complexes is detailed. In all the complexes the molar ratio between the cation and citrate or tartrate is 1:1. When the complexes are formed, the conductivities of the solutions are lowered and the extinctions are increased considerably. The complexes behave as if they are monobasic acids, and are neutralised by one equivalent of alkali at *p*H 6 to 8 to form more stable complexes, which are more suitable than the acidic complexes for use in retaining cations in solution during analytical procedures. When the neutralisation is carried out with sodium hydroxide, a remarkable increase in the extinctions of nickel and copper citrate solutions occurs. Addition of a second equivalent of sodium hydroxide to these solutions causes a decrease in the extinctions. The reaction caused by the addition of the first equivalent of sodium hydroxide to citrate complexes may be formulated as:



B. A.

Inorganic

Oxidation of Certain Metallic Hydroxides by a Current of Air. P. Fireman (*J. Amer. Chem. Soc.*, 1945, 67, 1447–1449)—(*a*) *Cobaltous hydroxide*—On drawing air through a suspension of freshly precipitated cobaltous hydroxide at room temperature in the presence of varying excess amounts of sodium hydroxide, grey-brown to dark brown products are obtained. The active oxygen contents, determined by absorption in a known excess of sodium oxalate solution and back-titration with standard permanganate, vary from 1·21 to 3·62 per cent., that of Co_6O_7 being 3·44. All the products after ignition in air between 260° C. and 270° C. show an active oxygen content tending to the value of 6·64 per cent., which corresponds to cobaltosic oxide, Co_3O_4. On drying at temperatures up to about 100° C., the degree of oxygen absorption shows no tendency to become constant.

In a boiling suspension, containing a minimum amount of sodium hydroxide, the degree of oxidation in the mother liquid is much greater, the active oxygen content of the air-dried product being higher than that of the ignited product, cobaltosic oxide.

(*b*) *Manganous hydroxide*—Using a boiling suspension, and in the presence of moderate excesses of either manganous ions or sodium hydroxide, the product obtained when air is passed through is always manganosic oxide. At room temperature, the oxygen content is slightly above that required for manganosic oxide, irrespective of the presence of moderate excesses of either manganese or alkali, and of the duration of the experiments.

Solid manganosic oxide absorbs oxygen when heated to 200° C. in air.

On prolonged ignition and after absorption appears complete, the composition of manganosic oxide seems to fluctuate by 1 part in 180, probably owing to variations in atmospheric pressure.

M. E. D.

Quantitative Determination of Tungsten, Molybdenum, and Vanadium alone and in the presence of Ferric Iron, with 8-Hydroxyquinoline. R. Niericker and W. D. Treadwell (*Helv. Chim. Acta*, 1946, 29, 1472–1483)—The *p*H values at which quantitative precipitation of 8-hydroxyquinoline complexes occurs are for ferric iron 2·8 to 11·2, for tungstic oxide 4·95 to 5·65, for molybdenum oxide 3·70 to 7·40, and for vanadium trioxide 2·7 to 6·1. The complexes may be weighed as such, or ignited to the oxides at temperatures below 500° C., or determined titrimetrically with bromate. All the complexes can be precipitated simultaneously at *p*H 5·0 to 5·5.

Method—Adjust to *p*H 7·3 with 2 *N* sodium hydroxide a solution containing tungstate, molybdate, and vanadate ions, and heat to boiling. Add a 4 per cent. solution of 8-hydroxyquinoline in 50 per cent. alcohol to an excess of 50 to 100 per cent. Of the three metals present the molybdenum complex precipitates at this *p*H; adjust to *p*H 5·0 to 5·5 by means of 2 *N*-acetic acid and phenol red to precipitate the three compounds together. Coagulate the precipitate by heating for 5 to 10 min., and filter rapidly; use the hot filtrate to effect quantitative transfer. Wash 3 or 4 times with a small quantity of hot water. Dry to constant weight at 120° to 140° C. Ammonium ions up to a concentration representing 1 to 5 ml. of concentrated aqueous ammonia have no effect. Precipitation is quantitative in the presence of 0·01 *M* phosphoric acid after neutralisation to *p*H 5·0, but is only partial in 0·1 *M*- and is completely arrested in 1·0 *M* phosphoric acid. Results are accurate to 1 in 250 on contents lower than 20 mg., and to 1 in 1000 on 50-mg. samples.

To facilitate low-temperature conversion to oxides it is necessary to remove the 8-hydroxyquinoline by heating with (*a*) oxalic acid or (*b*) ammonia. (*a*) Treat the complex in a Gooch crucible with 5 times its weight of moist, powdered oxalic acid, cover, and heat for 1 hr. at 150° C. in a tube evacuated by a water-pump. The oxide so obtained in the crucible can then be ignited at 480° C., at which temperature molybdenum oxide is not appreciably volatile. (*b*) Heat the complex in a stream of moist ammonia gas at 330° C. for 30 min., and then for the same time in a current of air. The oxide is ignited finally at 480° C. The conversion can be effected in a sloping tube closed with asbestos and heated in an air-bath at 330° C. Ammonia is drawn from a cylinder and moistened by passing through a wash-bottle containing concentrated aqueous ammonia heated to 80° C.; otherwise the conversion takes considerably longer. The efficiency of the conversion is good, especially for larger quantities.

Bromometric titration of the 8-*hydroxyquinoline complexes of tungstic oxide, molybdenum oxide, vanadium trioxide, and ferric iron*—The iron and vanadium complexes dissolve in dilute, warm phosphoric or hydrochloric acid, also slowly in cold, 2 *N* hydrochloric acid. The molybdenum and tungsten complexes are soluble only in warm concentrated phosphoric acid. Dissolve by pouring small quantities of pure, hot phosphoric acid through the filter, and dilute the solution so that it is 1·0 *M* with respect to phosphoric acid. Add 5 ml. of diluted hydrochloric acid (1+1), 1 g. of potassium bromide, and titrate with 0·1 *N* potassium bromate, using bright platinum wire to measure the potential. The end-potential occurs at eH=1·1 volt, but the potential at the beginning of the titration depends largely on the cation present. Vanadium shows a break at about 0·2 v., tungsten about 0·65 v., molybdenum about 0·35 v., and pure solutions of 8-hydroxyquinoline about 0·95 v. In 4 *M* phosphoric acid, 8-hydroxyquinoline can also be sharply titrated, but the increased acidity decreases the break in the curve.

Results show an accuracy of 1 in 600 on contents greater than 20 mg., and are slightly less accurate for contents down to 1·5 mg.

A visual determination may be made by adding a small excess of bromate and titrating back iodimetrically.

Weighing the oxides and 8-*hydroxyquinolinates in conjunction with the bromometric titration*—The ignited oxides dissolve readily in 2 *N* sodium hydroxide, molybdenum trioxide also in concentrated aqueous ammonia, but iron requires the addition of phosphoric and perhaps of hydrochloric acid. From the solutions so obtained the complexes can be again precipitated, dissolved in phosphoric acid, and titrated bromometrically. Hence indirect determinations of tungsten-molybdenum, tungsten-vanadium, and molybdenum-vanadium mixtures can be made, the weights of combined oxides and of combined hydroxyquinolinates, or the titration of the combined complexes and the weight of the combined oxides being utilised in the calculations. Results are accurate to 1 part in 250 on the figures given for tungstate-molybdate and vanadate-molybdate mixtures.

Direct determinations—Vanadium with tungsten and molybdenum—Precipitate and weigh the complexes, leach out the vanadium compound with 2 *N* hydrochloric acid, and wash the residue with a small quantity of water. Determine vanadium in the filtrate bromometrically, and molybdenum and tungsten in the residue by an indirect method.

Molybdenum and tungsten—Precipitate the molybdenum derivative in weakly alkaline solution, filter, add more reagent to the filtrate, acidify with 2 *N* acetic acid, and boil to precipitate the tungsten complex.

Tungsten and iron—Add 4 g. of Rochelle salt to the weakly alkaline tungstate solution containing some iron and treat with the reagent. The iron complex is precipitated, but the tungsten remains in solution even on acidification with acetic acid.

To permit determination of tungsten as well as of iron, the tartrate must first be replaced by a weaker complex-forming reagent such as glycerol. Treat the solution in 0·01 *M* phosphoric acid with 0·5 to 2 ml. of glycerol, and add sodium hydroxide solution to weak alkalinity. Add a 50- to 100-per cent. excess of the reagent, and boil to coagulate the precipitated iron complex, which can be determined either gravimetrically or with bromate. On adding more reagent to the filtrate, acidifying with acetic acid and boiling, the tungsten complex forms. Too large an excess of glycerol gives low results for iron, some of which remains for precipitation with the tungsten, but it can be extracted with cold 2 *N* hydrochloric acid.

Iron can also be removed from the combined and weighed complexes by extraction with cold, 2 *N* hydrochloric acid. Warm acid attacks the tungsten complex also, and *N* acid is inefficient, so careful control is necessary for good results.

Vanadium and ferric iron, and *molybdenum and ferric iron* may both be separated by the same method.

Since the complexes of divalent nickel, cobalt, and manganese, and of tervalent chromium are all more soluble than that of ferric iron, these may all be the more easily separated from tungsten, molybdenum, and vanadium.

Reduction by cadmium—By passage of a solution of sodium tungstate in *M* phosphoric acid through a cadmium reductor, the tungsten is reduced to the quinquevalent state, but when a solution of the tungsten 8-hydroxyquinolate in *M* phosphoric acid is similarly treated no reduction occurs. In a *M* phosphoric acid solution of the molybdenum complex, the molybdenum is reduced to the tervalent condition. M. E. D.

Extraction of Vanadium from Aqueous Solutions by *Iso*propyl Ether. J. J. Lingane and L. Meites, jun. (*J. Amer. Chem. Soc.*, 1946, 68, 2443–2447)—Although ether extraction has been recommended for the separation of iron from vanadium, few quantitative data on the behaviour of vanadium in this extraction are available. A study has now been made of the extraction of vanadium from aqueous solutions by *iso*propyl ether, and from the results obtained the optimum conditions for the separation of vanadium from ferric iron are defined.

No detectable amount of vanadium is extracted by peroxide-free *iso*propyl ether from solutions in diluted hydrochloric acid of quadrivalent vanadium, provided the duration of the extraction is short. If peroxide is present, or is formed during the extraction by the action of light on the ether, a vanadic acid is formed and this passes into the ether phase. The percentage of vanadium extracted from solutions in diluted hydrochloric acid, originally containing the vanadium in the quinquevalent state, is initially large, but decreases as the vanadate is reduced by chloride ion. For solutions of both quinquevalent and quadrivalent vanadium in the presence of hydrochloric acid, and for quinquevalent vanadium in the presence of sulphuric acid, the percentages of vanadium extracted at equilibrium, in diffuse daylight, rise with acid concentration from zero per cent. for 4 M acid to values greater than 5 per cent. at higher acid concentrations. The optimum conditions for the separation of vanadium from ferric iron by extraction are: the vanadium must be present in the quadrivalent state; the *iso*propyl ether must be peroxide-free; the extraction must not be prolonged for more than 10 min. in the presence of light; the concentration of hydrochloric acid should be between 7 and 8 molar, since the distribution coefficient of ferric chloride has then the most favourable value. A very sharp separation is obtained when these conditions are fulfilled.

The *iso*propyl ether can be freed from peroxide by distillation from dilute, aqueous potassium permanganate, and should be used at once. The quadrivalency of the vanadium can be ensured by evaporation of the iron-vanadium solution to dryness with concentrated hydrochloric acid. B. A.

Behaviour of Uranyl Solutions in a Mercury Reductor. E. R. Caley and L. B. Rogers (*J. Amer. Chem. Soc.*, 1946, 68, 2202–2204)—If a sufficiently high concentration of hydrochloric acid is present in the solution, the uranyl ion is reduced by the mercury reductor to the quadrivalent state. For a solution of uranium in 7 to 9 N hydrochloric acid reduction is rapid, but is incomplete by from 4 to 7 parts per thousand of uranium, depending on experimental conditions. The reduced solution is sufficiently stable, for analytical purposes, towards oxidation by air. A volumetric determination of uranium, in which the uranium was reduced in the mercury reductor, gives accurate results provided that a positive correction of 5 parts per thousand is applied to the final titration. Reduction in 18N sulphuric acid, or in uranyl acetate solutions containing sodium chloride, is slight.

The reductors used in the investigation were of the type described by McCay (*Ind. Eng. Chem., Anal. Ed.*, 1933, 5, 1). The general procedure was as follows. A 25-ml. portion of standard uranium acetate solution was pipetted into a reductor containing 250 g. of distilled mercury. A measured volume of acid, usually hydrochloric, was added, and the water phase diluted to 100 ml. The air above the liquid was displaced by carbon dioxide or nitrogen, and the reductor shaken at a known rate for a given time; in 7 to 9 N hydrochloric acid, reduction reaches a maximum in a few minutes. The reduced solution was filtered through a folded filter, the flask and filter being washed with air-free distilled water, and the filtrate and washings being collected in a measured volume of 0·1 N potassium dichromate. The excess of potassium dichromate solution was determined by back-titration with standardised ferrous sulphate solution, a platinum-tungsten pair of electrodes and a vacuum tube voltmeter being used to determine the end-point. B. A.

Fractional Separation of Hafnium and Zirconium by Means of Triethylphosphate. H. H. Willard and H. Freund (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 195–197)—This paper describes an extension of previous work on separations in which the precipitating ion was not added as such, but was formed slowly by hydrolysis in solution (Willard, *et al.*, *Ibid.*, 1937, **9**, 357; *J. Amer. Chem. Soc.*, 1937, **59**, 1190, 1197). By this method of precipitation a precipitate that is normally gelatinous may be obtained in a granular form that can easily be filtered and washed. Larsen *et al.* (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 512) have described a fractional separation of zirconium and hafnium in which the metal phosphates are precipitated with phosphoric acid under conditions so controlled that the gelatinous nature of the precipitate is overcome. The hydrolysis of triethyl phosphate in sulphuric acid solution is now employed successfully in this separation, the ester hydrolysing in a step-wise manner and precipitating zirconyl or hafnyl ethyl acid phosphate. If sufficient ester is added to precipitate only a fraction of the hafnium and zirconium, the hafnium concentrates in the precipitate.

The hafnium and zirconium salts were obtained from cyrtolite, the mineral being treated with concentrated sulphuric acid by the method of Larsen. To follow the separation two analyses were required, one for combined hafnia and zirconia and the other for the hafnia present in the combined oxides. Phosphate precipitates were decomposed by fusion with a mixture of sodium hydroxide and sodium peroxide and the hydroxides dissolved in sulphuric acid. Usually the combined oxides were first determined and then the oxides returned to solution by a potassium bisulphate fusion. The hafnium in this solution was determined by the following method. By a double precipitation with ammonia, followed by solution in 10 ml. of diluted hydrochloric acid (1 + 1), the hafnium and zirconium were transferred to hydrochloric acid solution. The liquid was boiled with 5 ml. of 30 per cent. hydrogen peroxide to destroy organic matter from the filter paper, and diluted to 200 ml. for precipitation of the selenites with 50 ml. of 20 per cent. selenious acid solution. The analysis was completed by the method of Schumb and Pittman (*Ibid.*, 1942, **14**, 512), in which the hafnium and zirconium are converted first to selenites and then to oxides. To remove silica from the combined oxides so obtained, 10 drops of concentrated sulphuric acid and 0·5 ml. of hydrofluoric acid were added and evaporated cautiously from the oxides. The sulphates were ignited to the oxides carefully and the ignition was completed at 950° C.

In the fractionation experiments, sufficient triethyl phosphate was added to solutions containing 0·1 g. mol. of combined oxides per litre of 6 *N* sulphuric acid to precipitate the desired fraction of the oxides as ethyl phosphates. A molar ratio of ester to oxides of slightly greater than 2 to 1 was required, to allow for side reactions. The mixture was boiled for 20 hours, cooled, filtered, and the precipitate washed with 2 per cent. sulphuric acid solution. Graphs are given showing the relation between initial mole fraction of hafnium, mole fraction of precipitate, and mole fraction of hafnium precipitated. In a series of fractionations for which data are given, 215 g. of combined oxides containing 16·0 weight per cent. of hafnia were enriched in five steps to 7·16 g. of combined oxides containing 91·1 weight per cent. of hafnia. B. A.

Analytical Chemistry of the Rare Earths. Active-oxygen Determination. G. L. Barthauer and D. W. Pearce (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 479–480)—Evaluation of the rare-earth metal content of a mixture of rare-earth oxides would be greatly simplified if a method were available by which the "active oxygen," the oxygen present in excess of that in the sesquioxide, could be determined. Such a method would also be useful in studying the composition of rare-earth oxides. The method of Bunsen for the determination of cations in higher valency states cannot be used in the presence of cerium dioxide, as there is only partial reaction between this compound and hydrochloric acid. Several modifications of the Bunsen method have now been tested, and a procedure using potassium iodide has been found to give satisfactory results for the amounts of active oxygen in pure cerium dioxide and in cerium dioxide lanthanum sesquioxide mixtures.

Method—The apparatus consists of a 125-ml. Kjeldahl flask fitted by a ground-glass joint to a water-cooled reflux condenser, the upper end of which is connected, by similar means, to a tube bent to a semi-circle and terminating in a Bunsen valve.

Procedure—Place a weighed sample of the oxide in the Kjeldahl flask, and add 20 ml. of 10 per cent. potassium iodide solution and 10 ml. of 12 *N* hydrochloric acid. Attach the condenser and Bunsen valve immediately, and immerse the valve in 80 ml. of a 4 per cent. potassium iodide solution, contained in a beaker. Heat the flask gently until the oxide has dissolved, cool, remove the vapour trap and, using the vapour trap solution, wash any iodine from the tube of the condenser into the flask. Transfer the solution to a 500-ml. Erlenmeyer flask, dilute to 350 ml., and titrate with 0·1 *N* sodium thiosulphate, starch being used as indicator. Perform a blank determination, using the same volumes of solutions and the same times of heating. The titration required for the blank should not exceed 0·5 ml.

An application of the method is illustrated by observations made on the ignition products of pure praseodymium oxalate. The composition of the oxide obtained by heating this compound in air at 955 ± 10° C. closely approximates to Pr_6O_{11}. B. A.

Agricultural

Determination of Quartz Content of Clay Fraction of Soils. L. de Leenheer and G. Waegemans (*Bull. Soc. Chim. Belg.*, 1945, **54**, 384)—The determination of the quartz content of clay fractions is important because the quartz forms an inert fraction which has no useful function as a soil colloid. Of the three most recent chemical methods of determination, that of Trostel and Wynne (*J. Amer. Ceram. Soc.*, 1940, **23**, 18) offers several advantages. In this method the clay fraction (0·5 g.) is fused with potassium pyrosulphate (10 to 15 g.) for 45 min. at 1000° C. A single fusion is sufficient, and the method is suitable for series determinations. According to the original authors, no correction need be applied to the results. Fusion may be performed in a quartz crucible.

The method has now been tested on fractions of pure, powdered quartz having definite particle size. The melt was dissolved in hot water, to which were added 12 g. of sodium hydroxide, and filtered through a filter crucible (porosity less than 2 μ. for finer fractions). The residue was washed with hot water, and then with dilute hydrochloric acid. The filtrate was found to contain appreciable quantities of dissolved silica, which was determined. The results show the claim of the original authors, that no correction is necessary, to be incorrect. The corrections required are as follows:

Fraction	Correction factor
below 2 μ.	1·064
2 to 5 μ.	1·044
5 to 10 μ.	1·039
10 to 20 μ.	1·029
20 to 50 μ.	1·027
50 to 100 μ.	1·022

When applied to the fractions below 2 μ., derived from a number of minerals, the following results were obtained:

Mineral	Quartz content Percentage
Montmorillonite (Geisenheim)	4·00
Muscovite (unknown origin)	3·08
Halloysite (Indianite)	1·85
Halloysite (Leoben)	3·53
Nontronite (Bavarian)	2·41
Kaolin (Meissen)	2·20

G. M.

Identification and Estimation of the Montmorillonite Group of Minerals, with Special Reference to Soil Clays. D. M. C. MacEwan (*J. Soc. Chem. Ind.*, 1946, 65, 298–304)—This paper describes the detection and estimation of montmorillonite (implying the whole montmorillonite group) by an X-ray diffraction method based on the formation of a montmorillonite-glycerol complex.

When the water-montmorillonite complex is employed as a means of detection of montmorillonite by X-ray diffraction, use is made of the 15 A basal reflection, which corresponds to two layers of water molecules between the structural sheets. In order to make this reflection as sharp and as strong as possible, careful control of the state of the base saturation and the hydration is necessary. Under the most favourable conditions, less than 5 per cent. of montmorillonite cannot be detected by this method, and there is liable to be ambiguity in identification because chlorites and vermiculites give reflections in the region of 14 to 15 A.

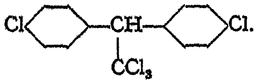
The glycerol-montmorillonite complex, which is formed by addition of glycerol to the clay (not necessarily free from water), has the following advantages over the water-montmorillonite complex:—(1) Intense first-order basal reflection at 17·7 A. This reflection, and the higher order basal reflections, do not interfere with reflections from other likely minerals. (2) Since glycerol is miscible with water, it is easy to change from a water system to a glycerol system. (3) Spacings and intensities of the glycerol-montmorillonite basal reflections are unaffected by wide variation in the water content. (4) The complex is extremely stable, even in an evacuated camera.

A list of basal reflections and intensities due to the glycerol-montmorillonite group is reproduced, together with other reflections likely to occur on soil clay photographs.

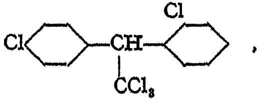
A discussion is given on the possibility of other minerals giving the 17·7 A reflection, and includes the question of distinction between montmorillonite and hydrous micas. Experience so far shows that all minerals giving the 17·7 A reflection when treated with glycerol, may be provisionally assigned to the montmorillonite class and those giving a 10 A reflection, to the mica class.

For detecting small quantities of montmorillonite, the oriented aggregate technique is used, the specimen being made by evaporating a suspension to dryness. An evacuated camera having an efficient collimating system should be used. By this method, 1 per cent. of montmorillonite can be detected, without ambiguity, in mixtures containing any other clay minerals. The extension of the method to rough quantitative analysis of montmorillonite clays is also described. E. G. S.

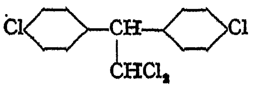
Chemical Investigations of the Insecticide DDT and its Analogues. Part I. Reactions of DDT and Associated Compounds. J. Forrest, O. Stephenson, and W. A. Waters (*J. Chem. Soc.*, 1946, 333–339)—Pure DDT prepared by Zeidler's method (*Ber.*, 1874, 7, 1181) by condensing chlorobenzene with chloral or chloral hydrate in concentrated sulphuric acid crystallises from solvents in long, white needles with m.p. 108° to 109° C., but the uncrystallised technical product prepared by the same process on the large scale is a white powder with a lower setting point and inferior physiological potency. The most potent insecticide in crude DDT is 1:1:1-trichloro-2:2-di-(4-chlorophenyl) ethane



By careful crystallisation and distillation under reduced pressure of the residues in the mother liquor from the crystallisation of crude DDT from alcohol, there was obtained 1:1:1-trichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane,

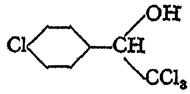


or *iso*-DDT, which is almost devoid of insecticidal activity. Technical DDT normally contains over 90 per cent. of these isomers in the approximate ratio of 4 of pure DDT to 1 of *iso*-DDT. A common impurity in technical DDT is 1,1-dichloro-2:2-di-(4-chlorophenyl)ethane



or DDD. This has about one-third the insecticidal activity of true DDT, and can be prepared by condensing chlorobenzene with either dichloroacetal or dichloroacetaldehyde; it is evidently formed from these impurities in the technical chloral used in the manufacture of DDT. It has the effect of lowering the setting point of DDT to a value not commensurate with the loss of physiological activity.

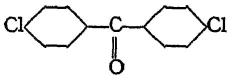
It is characteristic of DDT and the above-mentioned associated compounds that, by treatment with alcoholic alkali, they lose one molecule only of hydrochloric acid, yielding very stable ethylenes, which are resistant to further alkaline hydrolysis. Thus extraction with benzene or petroleum, hydrolysis of the washed, salt-free extract with alkali, and subsequent estimation of chloride can be made the standard method of analysis of DDT for detecting gross impurities, as well as for its estimation in insecticidal sprays, or powders, or upon fabric. This method, however, does not differentiate between pure DDT and the physiologically inert *iso*-DDT. Neither can it be used as a sensitive test for the presence of DDD since the hydrolysable chlorine content of DDD (11·1 per cent.) is only slightly more than that of DDT (10·0 per cent.). Estimation of the total chlorine (44·3 per cent. for DDD and 50·1 per cent. for DDT) is more indicative of this contamination, though it is impracticable as a rapid routine method. High hydrolysable chlorine figures (over 10·5 per cent.) indicate appreciable contamination with the half-condensation product 1:1:1-trichloro-2-hydroxy-2-(4-chlorophenyl)ethane



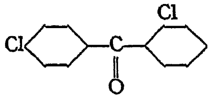
because this on boiling neutralises nearly four equivalents of alkali, liberating three equivalents of chloride per gram-molecule. Crude DDT may contain about 3 per cent. of an oily substance consisting mainly of this impurity.

A study of the products of oxidation of the substituted ethylenes formed by hydrolysis of DDT led to the following general procedure for the analysis of technical DDT.

Procedure—Dissolve 25 g. in 200 ml. of warm alcohol and heat under reflux for 0·5 hr. with 8 g. of sodium hydroxide dissolved in 50 ml. of water. Remove the excess of alcohol on the water-bath, dilute the residue with water, and acidify with hydrochloric acid. When cold, decant the aqueous layer from the gummy residue, extract it with chloroform, and evaporate the extract to dryness. Dissolve the brown gum, together with the residue from the chloroform extract, in 75 ml. of hot, glacial acetic acid and add gradually a saturated solution of 50 g. of chromic acid. Reflux the mixture for 4 hr., dilute while still hot with 500 ml. of water, and allow to cool. Extract the cold liquid with chloroform, and wash the extract four times with small amounts of diluted aqueous ammonia, and finally with water. Evaporate the alkaline aqueous layer to small bulk, filter, acidify, and collect and weigh the precipitate (Y) in a Gooch crucible. Dry the washed chloroform extract over calcium chloride and collect the ketone mixture (X) quantitatively by evaporation of the filtered solution. Heat the residue above its m p. to remove traces of chloroform, cool, and weigh. Determine the setting point of the mixture, which consists of the dichlorobenzophenones



and



The precipitate (Y) consists of *p*-chlorobenzoic acid derived from the half-condensation products. A wide range of samples of technical DDT yielded ketone mixtures (X) of setting point 132° ± 2° C. corresponding to a content of 26 per cent. of the 4-2′-isomer. The composition of the ketone mixture may be determined by means of a curve showing the relation between the setting point of a mixture of the two ketones and the proportion in the mixture of one of them. Most technical samples of DDT have setting points above 75° C., and the figures calculated from the analytical results fall between the following results found with two samples:—Setting point, 91°, 80° C.; DDT per cent., 76, 65; hydrolysable chlorine per cent., 10·2, 10·6; total chlorine per cent., 49·2, 48·9; DDT compounds per cent. from weight of ketones, 88, 85; setting point of ketones, 133°, 134° C.; half-condensation products per cent., 3·2, 2·6; probable per cent. of DDD, 2, 16; maximum *iso*-DDT + DDD, 29, 25.

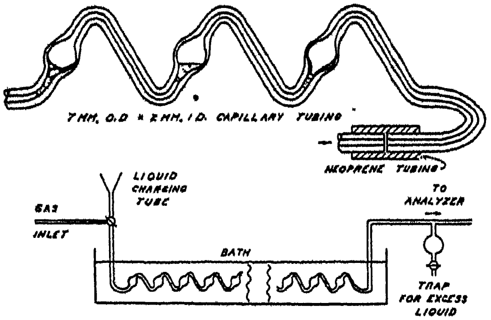
Other reactions of DDT and its analogues have been studied. When DDT, *iso*-DDT, and DDD are nitrated, the nitro-groups enter the aromatic nuclei in the 3 and 5 positions. The reduction of DDT affords an example of a reversed benzil-benzilic acid transformation, DDD and 4:4′-dichlorostilbene being produced. A. O. J.

Gas Analysis

Identification of Sulphur Compounds in Gas Mixtures. C. S. Oldach and E. Field (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 669–672)—A method of identification and determination of organic sulphur compounds at a concentration of a few parts per million is based on solubility differences in inert solvents, combined with a highly sensitive analytical method (*Ibid.*, 1946, **18**, 665, 668). The method can also be applied to any class of compounds for which a sensitive analytical method is available.

From Raoult's Law it can be deduced that $n=P/p_T$, where P is the total gas pressure, p_T is the vapour pressure of the pure solute at temperature T, and n is the minimum quantity of gas required to saturate one g.-mol. of liquid with the solute. Experimental determination of n reveals the solubility or vapour pressure of the solute, which usually will identify the organic sulphur compound. The relationship between the two phases holds both when a liquid is being saturated by absorption of a component from a gas, or a gas is being saturated by stripping a compound from a liquid. To determine the minimum quantity of gas saturating a given volume of liquid, an efficient saturator, such as the one described, is essential.

Method. Apparatus—A suitable saturator is constructed from twenty 60-cm. lengths of 2 × 7 mm. capillary tubing containing 200 enlargements as shown in the figure, the sections abutting in Neoprene joints. White oil, the inert solvent, is introduced through the liquid charging tube and distributed by passing hydrogen, thus obtaining 200 pools of solvent. The whole is immersed in a thermostat bath giving a temperature regulation to ± 0·15° C.; any liquid carried out of the saturator is caught in a trap. The exit gases may be analysed by conversion to hydrogen sulphide over heated alumina, followed by spectrophotometric determination (*loc. cit.*).



Absorption run—Distribute a known volume of sulphur-free solvent through the saturator, and adjust to a known temperature. Remove hydrogen sulphide from the sample gas by means of a Thylox unit, and sample the gas by filling a stainless steel cylinder under pressure. Pass the hydrogen-sulphide-free gas into the apparatus at a rate of 0·005 cu.ft. per min, and analyse the emergent gas at frequent intervals (*loc. cit.*, p. 668). Plot the sulphur contents found against the volume of treated gas. A break in the curve indicates absorption of a new sulphur compound; the volume at which it occurs serves to identify the compound, and the magnitude of the break shows its concentration.

Calculation of the "break volume"—For carbon disulphide: $P = 1$ atmosphere, p_T is 0·308 atmospheres at 14° C., thus $n = 3{\cdot}25$ g.-mols. of gas to saturate 1 g.-mol. of oil with carbon disulphide. Using 48 ml. of solvent oil of density 0·77 and average molecular weight 154, a total of 0·000529 pound-mols. of oil, the amount of gas required is $3{\cdot}25 \times 0{\cdot}000529 = 0{\cdot}00172$ pound-mol. Under wet meter conditions this gives a calculated break volume of $0{\cdot}00172 \times 359 \times \frac{295}{273} \times \frac{760}{741} + 0{\cdot}02$ cu.ft. of free space in the absorber = 0·70 cu.ft. The amount of carbon disulphide is thus shown by the magnitude of the break at 0·70 cu.ft., at standard temperature and pressure. The similarity of the vapour pressures of hydrogen sulphide and carbon disulphide makes their distinction difficult, thus hydrogen sulphide is determined separately by absorption in cadmium acetate, acidification with acetic acid and iodimetric titration (Shaw, *Ibid.*, 1940, **12**, 668). For the determination of small concentrations of gases less volatile than carbon disulphide a stripping run is necessary, the precision being better than ± 0·1 grain per 100 cu.ft. when the concentration of sulphur in the gas drops below 1 grain per 100 cu.ft.

Stripping run—Saturate a 100-ml sample of the solvent with the gas by bubbling it through at 70 cu.ft. per hr. for 2 hr. at −5° C. in a flask fitted with a sintered-glass sparger. Transfer about 50 ml. to the saturator and distribute by means of a stream of hydrogen, avoiding all contact with air. Strip then by passing hydrogen through at 0·007 cu.ft. per min. at a temperature of about 20° C.; analyse the gas and plot a graph as before. A temperature correction must be made to allow for the difference in temperature between saturating and stripping, and is given by the ratio of the vapour pressures at the saturating and stripping temperatures. The more volatile constituents are removed before accurate analyses can be made, but accurate results are obtained for the less volatile constituents. A complete analysis usually requires both an absorption and a stripping run.

Sources of error—The accuracy of the method has not been tested on known samples, but the results obtained agree with previous experience of industrial gases. Certain compounds are unstable to white oil, oxygen, and metal walls, so determinations should be made rapidly. Any inert solvent that is not highly volatile or too viscous may be used. In the presence of a high concentration of a compound, the solvent will increase in volume and corrections must then be applied for variations in the solubility. The accuracy of the stripping run is increased by saturating at a temperature below that used in the stripping. Adsorption of sulphur by alumina during the catalytic reduction of organic sulphur compounds to hydrogen sulphide is minimised by using only 1 ml. of the catalyst.

Sulphur content - gas volume curves obtained in identifying and determining the sulphur compounds present in coke-oven gas are presented. M. E. D.

Microchemical

Combined Micro-gravimetric and Titrimetric Micro-determination of Thallium in Toxicological Material. J. F. Reith and K. W. Gerritsma (*Rec. Trav. Chim.*, 1946, 65, 770–780)—*Preparation of the material*—For urine, evaporate 500 ml. acidified to Congo red paper to about 70 ml., add 15 ml. of concentrated hydrochloric acid and small amounts of potassium chlorate to the warmed liquid until it is of a clear yellow colour and smells distinctly of chlorine. Cool, filter, and collect the filtrate in a 100-ml. cylinder and rinse the filter with a little water. For faeces, rub the material with water to a thin paste and take a known amount of the homogeneous preparation. Grind viscera to a homogeneous paste in a meat grinder. Use a maximum amount of 60 g. of faeces of normal consistency and 60 g. of viscera for the determination. To the material in a tall, 300-ml. beaker add an equal weight of 4 *N* hydrochloric acid, 10 mg. of manganese (*e.g.*, 10 ml. of 0·1 *N* potassium permanganate) as catalyst, and about 1 g. of potassium chlorate. Heat to the boiling-point and, as the liberated chlorine is consumed, add 0·5- or 1-g. portions of potassium chlorate until the liquid has a clear yellow colour, with a heavy sediment of chlorinated fats, and no longer becomes brown after being heated for a further 30 min. on the water-bath. Filter the liquid, which must retain an odour of chlorine, heat the insoluble portion with a little 4 *N* hydrochloric acid and potassium chlorate, pour the liquid through the filter and rinse with a little water. For hair and nails, destroy the organic matter with nitric acid and a little sulphuric acid, dilute with water, increase the hydrochloric acid concentration to 2 *N* and introduce a little free chlorine by means of a hot solution of potassium chlorate in 4 *N* hydrochloric acid.

Extraction—Transfer the clear liquid to a separating funnel and confirm the presence of free chlorine by means of starch-iodide paper. Shake vigorously with an equal volume of ether, separate the aqueous layer and transfer the ether layer, which contains thallium trichloride, to a second separating funnel. Add so much sulphur dioxide water (1 to 2 ml.) that, after vigorous shaking, the aqueous layer no longer reacts with starch-iodide paper. Adjust the volume of the aqueous layer to 5 ml., shake the mixture vigorously and separate the aqueous layer containing thallous chloride into a dish. Extract the ether layer again with about 2 ml. of water and combine this extract with the first. Shake the ether again with the prepared sample material in the first funnel, add a little potassium chlorate in hydrochloric acid, if necessary, to ensure the presence of free chlorine and shake the mixture vigorously. Transfer the ether layer to the second separating funnel, treat it with sulphur dioxide water and proceed as already described, combining the final aqueous extracts with those in the dish. Repeat the extraction procedure a third time with the same ether. Evaporate the combined aqueous extracts containing thallous chloride on the water-bath until a black syrup remains. Transfer this by means of a few drops of nitric acid to a 50 × 18 mm. Pyrex glass tube, add 0·2 ml. of concentrated sulphuric acid, and destroy the organic matter in the usual way, using the necessary small additions of nitric acid to complete the rinsing of the dish. The destruction is complete when, even after boiling, the liquid remains colourless or light yellow.

Precipitation and weighing of the thallous iodide—When the amount of thallium ranges from 0·01 to 2 mg. carry out the precipitation in Pyrex tubes of 2 ml. capacity (45 × 10 mm.). Dilute the oxidised extract with 0·8 ml. of water, cool, and filter with suction through a micro-filter of sintered glass (diameter, 9 mm.). Collect the filtrate directly in the precipitation tube and rinse several times with drops of water until the final volume is 1·8 ml. Add 0·1 ml. of freshly prepared, saturated, filtered sodium sulphite solution and mix with a glass rod. Then add 0·2 ml. of filtered, 10 per cent. potassium iodide solution and mix. An orange-yellow precipitate indicates thallium. Rinse the rod and allow the covered tube to stand for 12 to 18 hr. in the dark. Centrifuge the tube for 5 min. at 1500 r.p.m. and pour off the supernatant liquid along a glass rod. Wash the inner wall of the tube and the precipitate in the usual way with 2 ml. of 50 per cent. alcohol, stirring the precipitate with a glass rod which is then rinsed with the alcohol. Centrifuge and decant again and repeat the washing procedure with 90 per cent. alcohol. Rinse the outside of the tube with distilled water and alcohol, heat for 2 hr. at 100° to 105° C., cool, keep in the microbalance case for 5 hr., and weigh. Treat a second tube, serving as tare, in the same way with 2 *N* sulphuric acid and 50 and 90 per cent. alcohol. After removing the thallous iodide by solution in acetic acid and bromine (*infra*), rinse the tube, heat, and weigh as already described and record its weight as that of the empty tube. To convert the thallous iodide to thallium, multiply by 0·616.

Titration of thallous iodide—Treat amounts of thallium ranging from 10 to 250 μg. as follows. Place in the dry tube containing the precipitate, which must be free from alcohol, 0·1 ml. of glacial acetic acid and, from a thin pipette, 1 drop of bromine. Shake momentarily every 5 min. until no solid particles remain visible and then allow to stand for 15 min. Rinse the contents of the tube with water into a 25-ml. Erlenmeyer flask using not more than 2 ml. of water. Heat the flask carefully until the liquid is light yellow and allow it to cool. Add 2 *M* sodium formate until the solution is colourless and then an excess of 0·2 ml. Mix carefully, allowing the liquid to moisten the walls, and set the tube aside for 5 min. Add successively 2 ml. of 30 per cent. sodium chloride solution, 1 drop of 10 per cent. potassium iodide solution, 0·2 ml. of 4 *N* sulphuric acid and 5 drops of 0·2 per cent. starch solution. Titrate with 0·01 *N* sodium thiosulphate, each ml. of which is equivalent to 255·5 μg. of thallium. With amounts of thallium ranging from 250 to 5000 μg., proceed in the same way with suitable increases in the volumes of the reagents used. A blank titration should always be performed and, as a check on all the reagents, a blank analysis should be made on 100 ml. of distilled water. Since thallium salts are rarely pure, the thallium content of thallium solutions used as standards should be determined by titration in the manner described after treatment with acetic acid and bromine. Lead, mercury, copper, arsenic, antimony, bismuth, and iron do not interfere with the method. A. O. J.

Thiocyanate Complex as a Means of Extracting Cobalt before its Micro-determination by Other Methods. N. S. Bayliss and R. W. Pickering (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 446–448)—The complex thiocyanate of cobalt and ammonia is soluble in organic solvents, and can be used for the colorimetric determination of small amounts of cobalt, but not for the very small amounts present in biological samples. Iron, nickel, copper, and zinc form similar complexes; that of iron is relatively insoluble in ethereal solvents, and the others do not interfere with the final determination of cobalt by the nitroso-R-salt method.

Procedure—Dissolve a sample containing 10 to 20 μg. of cobalt in enough hydrochloric acid to furnish an excess of 5 ml. of 6 *M* acid. Add 20 ml. of 60 per cent. ammonium thiocyanate solution, previously purified by extraction with a 0·1 per cent. solution of dithizone in chloroform and washing with chloroform and amyl alcohol. Then add *M* ammonium citrate until the red colour due to ferric thiocyanate just disappears. Dilute with water to 50 ml., and saturate with 4 ml. of ether. Extract the aqueous solution with three 20-ml. portions of a mixture (35+65) of amyl alcohol and ether to remove the cobalt from the aqueous phase as $(NH_4)_2Co(CNS)_4$, the iron not being extracted. Shake the combined extracts with two 20-ml. portions of 2 *M* aqueous ammonia to return the cobalt to the aqueous phase. Evaporate the ammonia extracts to dryness, and then evaporate again to dryness with 20 ml. of 6 *M* nitric acid to decompose the thiocyanate. Neutralise the residue with 2 *M* aqueous ammonia and evaporate to dryness. Determine cobalt in the residue by the method of Marston and Dewey (*Australian J. Exptl. Biol. Med. Sci.*, 1940, **18**, 343), by adding 5 ml. of water, 5 ml. of *M* ammonium citrate, and 0·5 ml. of a 1 per cent. solution of nitroso-R salt. Heat the solution on the water-bath for 5 min., add 5 ml. of concentrated nitric acid, and heat 5 min. longer. Cool, add 2 ml. of a saturated solution of bromine in water, and boil to expel the excess of bromine. Determine the cobalt content by comparisons with standards of known content prepared from standard cobalt sulphate solution and ammonium sulphate.

The optimum solvent concentration was found experimentally to be a mixture (35+65) of amyl alcohol and ether, the extraction of cobalt being about 85 per cent. on a single treatment of the aqueous phase at a *p*H of approximately 3·4. Efficient extraction requires a minimum thiocyanate concentration of 20 per cent. in the aqueous phase, half that value giving an efficiency too low for analytical use.

Since biological samples always contain an iron content large relatively to that of cobalt, the iron complex must be suppressed when determining cobalt. It is found that ammonium citrate destroys the ferric thiocyanate coloration at a *p*H value close to that at which maximum extraction of cobalt occurs; iron thus forms a useful indicator which does not interfere with the final colour comparison, as do many organic indicators.

Thiocyanate is conveniently removed by 6 *M* nitric acid, stronger acid resulting in a reaction that is too violent; incomplete removal causes a violent reaction during the nitration of the excess of nitroso-R salt. M. E. D.

Physical Methods, Apparatus, etc.

Particle Size by Spectral Transmission. E. D. Bailey (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 365–370)—A method is described for determining two-parameter size distributions from transmission measurements on suspensions in the visible and near infra-red, based on an empirical relationship between scattering, particle size, refractive index, and wavelength.

Suspensions are prepared by means of a pestle and mortar, and the concentration is adjusted to give about 20 per cent. transmission with blue light. Transmissions are measured at 14 points over a wavelength range of 0·4–2·0 μ., a monochromator and photo-cell or vacuum thermopile being used. The results are plotted and compared with a standard family of curves computed from the distribution law of Lansing and Kraemer (*J. Amer. Chem. Soc.*, 1935, **57**, 1369). The experimental curves are evaluated in terms of r_0, the radius value of the maximum of the distribution, B, the non-uniformity coefficient which defines the spread of the curve, and a percentage expressing the extent to which the selected distribution law is obeyed. The method of analysis is rapid, requiring only 2 hours, but is limited to non-absorbing materials and to particle sizes for which optimum scattering occurs in the wavelength ranged used (r_0 < about 1 μ.).

The results are in fairly good agreement with those obtained with the ultracentrifuge.

Comparisons of particle-size distributions in samples of anatase, bentonite, rutile, and zinc oxide are given. B. A. S.

Internal Standard Method of Spectrographic Analysis as applied to the Determination of Lead in High-Purity Zinc. L. Griffith and J. N. Kirkbride (*J. Soc. Chem. Ind.*, 1946, **65**, 39–48)—An investigation has been made into the fundamental features of the internal standard method of spectrographic analysis, the controlled direct current arc-method for the determination of lead in high-purity zinc with bismuth as the internal standard being used as the example. The working conditions for this investigation were standardised, and, using the average deviation as a criterion, a precision of 2 per cent. over the lead range 0·025–0·003 per cent. was reached.

The principal error arises from the failure of the Eastman "33" plates used to obey the reciprocity law. Reduction of this error can only be achieved by using plates having little reciprocity failure, since the use of the direct-current graphite-arc precludes utilisation of a relative intensity calibration. Allied to this, is the error due to the intermittency effect, which can be reduced by speeding up the stepped sector.

The second error is the Eberhard effect, which occurs when there is a large range of density over a small distance of plate; this gives rise to local differences in concentration of the developer, and produces an erroneous density-intensity curve. The best solution to this problem seems to be increased agitation of the plate during development.

Bismuth is not an ideal internal standard since the lead line 2833·07 A and the bismuth line 2897·98 A are not a perfectly homologous pair. Although this constitutes the third source of error, it is doubtful whether any improvement is possible.

The error involved by the use of the direct current arc is certainly less than the errors already enumerated. D. A. P.

Comparison of Optical and Electron Microscopy of Pigments. D. L. Tilleard and N. D. P. Smith (*J. Soc. Chem. Ind.*, 1946, **65**, 261–264)—The high resolving power of the electron microscope enables problems relating particle size and shape to pigment colour, wetting, and other characteristics to be investigated. The small numerical aperture (0·001 radian) employed and the resultant depth of focus (10 μ. as opposed to about 0·4 μ. with N.A. 1·25) enables useful stereoscopic micrographs to be obtained.

Most pigments can be dispersed in a mixture of linseed stand oil and white spirit by grinding on a glass plate with a spatula. A drop of this dispersion is placed on a supporting film prepared by allowing a drop of a 2 per cent. solution of nitrocellulose (high nitrogen content; medium viscosity) in amyl acetate to fall on the surface of clean water. When the spirit has evaporated, selected portions of the film are attached to supporting screens and the excess oily matter is removed with white spirit. *Comparison of optical and electron micrographs*—The electron micrographs of various pigments frequently show particles whose size is below optical resolution. The detail of the shape of many particles in the region of uncertain optical resolution (between 0·5 and 0·25 μ) is disclosed. In the electron micrograph some particles of red earth pigment appear grey, indicating that they are extremely thin; others suggest a hexagonal shape similar to that observed in micrographs of clay. The micrograph of "ferrite" yellow shows rod-shaped particles, 0·03 to 0·1 μ. wide and about 0·5 μ. long, whereas another synthetic iron oxide pigment, Turkey Red, shows rounded particles less than 0·1 μ. diameter. The varying sizes and shapes of zinc oxide pigments to be seen in the electron micrographs reproduced may be related to methods of manufacture; the electron microscope reveals the regular crystalline shape of the particles of all the zinc oxides examined. Differences in samples of lead chrome pigments are also seen, and may be related to the lead sulphate content of the pigment, although the tendency to change from monoclinic towards orthorhombic habit with an increase in lead sulphate content cannot be deduced from the micrographs. When interpreting electron micrographs it is necessary to remember that the particles seen may be only the smallest in the pigment; particles commensurate with the size of the whole field of the microscope may be neglected; and that the quantity of material in the whole field is very small and, except in special circumstances, quantitative conclusions are not justified. G. A. B.

Oxide Films Formed on Alloys at Moderate Temperatures. Electron Diffraction and Electron Microscope Study. E. A. Gulbranson, R. T. Phelps, and J. W. Hickman (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 640–652)—This paper describes the study of oxide films by reflection and transmission methods of electron diffraction, in addition to electron microscope studies. The alloys studied were those consisting principally of iron, cobalt, nickel, and chromium, and are typical commercial protective, refractory, magnetic, and sealing alloys. A miscellaneous group of experimental alloys has also been included.

Electron diffraction results—(1) Iron and chromium ions diffused more readily to the surface of the oxide film than the other metal ions. (2) Stratification of oxides occurred even for films 100 to 300 A thick. (3) Chromium sesquioxide was always observed in the oxide films on the protective or refractory alloys examined; where stratification occurred, chromium sesquioxide appeared to be in contact with the substrate. (4) Nickel monoxide was never observed on the surface of the film, but sometimes occurred in the body of the film; this may indicate a low diffusion rate for the nickel ion. (5) Solid solution of two simple oxides to give the spinel-type structure may occur. (6) Except for iron and chromium, metals constituting not more than 5 per cent. of the alloy did not occur as simple oxides on the outer surface, but appeared to form spinels in the body of the film. (7) Simple oxides of cobalt, manganese, silicon, vanadium, and tungsten were not observed.

Electron microscope results—(1) The thin oxide films consisted of a continuous film of oxide crystals 100 to 1600 A in size. (2) The oxide films were not of uniform density and the crystals had irregular shapes. (3) Longer oxidation time increased average crystal size. (4) Crystals on non-protective alloys such as mild steel, at 250° C., were of the same size as those found on protective and refractory alloys at 600° C. (5) At a given temperature, a correlation may exist between crystal-size of the oxide and the protective quality of film. (6) Thickness of boundary zone between crystals in the mosaic structure was of the order of 50 A.

Electron diffraction data, and numerous electron micrographs of oxide films on stainless steels, nichrome, cobalt-iron, chrome-iron, silicon-iron, manganese-iron, Iconel, and mild steel are reproduced. E. G. S.

Determination of Extinction Corrections in Infra-red Analysis of Gaseous Hydrocarbon Mixtures. J. H. Lee (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 659–661)—The usual procedure in the infra-red analysis of gaseous hydrocarbon mixtures involves the calculation of various corrections to be applied to the observed extinction coefficients of the mixtures. The sources of error for which corrections must be applied are, energy scattered within the instrument, the structure of the absorption band, and inter-molecular action. By use of the relation, $E_c = E_0(1+\alpha E_0)$, where E_c and E_0 are, respectively, the corrected and the observed values of log. I_0/I, and α is a constant for the particular instrument, gas, and absorption band concerned, one method of correction, allowing for the errors caused by the several factors, can be applied. When molecular interaction has an appreciable effect on absorption, addition of a diluting agent such as air is desirable if the proposed method of correction is to be used. Details of the method for calculating corrections to be applied directly to observed extinctions are given. B. A.

Preparation of Powdered Materials for Electron Microscopy. M. C. Schuster and E. F. Fullam (*Ind. Eng. Chem. Anal. Ed.*, 1946, 18, 653–657)—The aggregates in powdered materials must be adequately dispersed to furnish electron micrographs suitable for statistical particle-size distribution analysis. Specimens are mounted on thin (10 to 30 mμ.), electron-transparent, resin films which are in turn supported on 0·3-cm. diameter metal screens having perforations of 20 to 100 μ. Deflocculation may often be brought about by choice of a mounting medium with suitable wetting properties. A common method of film formation is to drop a small amount of resin solution on water and allowing it to spread and dry. Screens may be placed on it and picked up from beneath with an annular tool. The thickness of the film is determined by the concentration and spreading coefficient of the solution and the volatility of the solvent. A purified nitrocellulose dissolved in amyl acetate is in general use. Mercury is, theoretically, an excellent substrate, but the manipulative difficulties are considerable. Tougher films are formed by Formvar 15/95, Grade E, a polyvinyl formal polymer. A solution (0·2 to 0·4 per cent. by weight) in ethylene dichloride, which should be stored in a brown bottle, is spread evenly on to a microscope slide and held vertically in an atmosphere of the solvent to dry. The film is scored round the edge with a needle and floated on water by inserting the slide obliquely. Breathing on the dry slide and application of additional coats of solution round the edge facilitate stripping. Films showing interference colours are too thick.

Powders that can be dispersed in a volatile liquid may be mounted by placing a drop of suspension on a filmed screen and allowing it to dry. An adaptation of the turpentine dispersion method in optical microscopy has been devised. The pigment in a drop of turpentine is rubbed out with a glass rod on a microscope slide until dry. The dispersion is flooded with resin solution and floated on to water. Screens are placed on areas selected under an optical microscope. This method is most suitable for uniform powders 0·5 μ. and greater. Many excellent dispersions may be prepared by working a pigment suspended in a viscous resin solution on a plate glass table with a stiff, stainless steel spatula. The proportion of specimen to resin for a suitable concentration of particles in the field depends on particle size and can only be found by experience. Substances too soft to rub out mechanically may be made brittle by freezing with solid carbon dioxide.

Dispersion by supersonic and other vibratory methods has not been entirely satisfactory. A small pile of dry powder placed on a filmed slide may often be dispersed by passing a spark from a Tesla coil across the under side of the slide. Mechanical mills for dispersion have the disadvantage of requiring a large sample. The resin containing a dispersed material is made into a thin film by methods similar to those already described for clear films. Alternatively, the suspension may be evaporated to the consistency of thick cream and drawn down on a slide with a spatula by a series of short strokes. Samples prepared in this way exhibit fewer holes in the film. Large-size powders (1 to 10 μ.) may be mounted, using dibutyl phthalate or a high-boiling hydrocarbon as suspending medium and drawing down as before on a film-covered slide.

Electron micrographs of different types of mounts showing the particle size and shape of iron oxide, antimony oxide, chromium oxide, zinc oxide, calcium carbonate, and ultramarine are reproduced.

TABLE I. RESINS AND SOLVENTS

	Recommended solvents			
Resin	For dispersion[a]	For mounts cast on glass[b]	For mounts spread on water[c]	For mounts drawn down on glass[c]
Nitrocellulose (Parlodion)	Cellosolve acetate	Methyl acetate Ethyl acetate	Methyl acetate Ethyl acetate Amyl acetate Cellosolve acetate Octyl acetate	Ethyl acetate Amyl acetate
Formvar 15/95	Dioxan plus a few drops of dimethyl dioxan Nitroethane	Ethylene dichloride Nitroethane	Dichloroethylene Propylene dichloride Ethylene dichloride plus 0·1 per cent. of oleic acid Nitroethane 1:1 Dichloro-2-nitroethane	Dioxan Nitroethane
Polystyrene	Xylene	Ethylene dichloride Toluene	Benzene Toluene Xylene	Benzene Toluene
Methyl methacrylate or cellulose acetate	Methyl amyl ketone Methyl ethyl ketone plus 10 per cent. of *iso*phorone	Acetone	Methyl ethyl ketone Methyl amyl ketone *Iso*phorone	Methyl ethyl ketone

[a] 10 to 20 per cent. solutions, approximately. [b] 0·1 to 0·5 per cent. solutions, approximately.
[c] No fixed concentration; suspension allowed to dry to suitable consistency.

G. A. B.

Reviews

PENICILLIN: ITS PROPERTIES, USES AND PREPARATIONS. Published by direction of the Council of The Pharmaceutical Society of Great Britain. Pp. viii + 199 + xv. London: Pharmaceutical Press. 1946. Price 10*s*. 6*d*.

The publication simultaneously in *Nature* and *Science*, at the end of 1945, of what was then suggested as, and is still held to be, the chemical formula of penicillin, cleared away much of the mystery from its behaviour, which had previously appeared somewhat freakish. A book such as the one before me, wherein pharmacists in the first instance, but medical men and chemists as well, are given a full, straightforward, yet not over-simplified account of the penicillins, their properties and uses, could hardly have been written before the disclosure, not previously allowed for reasons of national security, of their chemical structure, on which their chemical properties depend and can be to a considerable extent explained. But we are no nearer with the penicillins than with most other drugs to understanding how biological behaviour is determined by chemical composition.

It appears certain that the biggest foe to penicillin as an entity—and, what is more to the practical point, to its anti-bacterial activity— is water, for the irreversible hydrolytic breakdown of the -CO-NH- link is, besides naturally being accelerated by heat, catalysed by hydrogen and hydroxyl ions and also by the bacterial enzyme penicillinase. The other weak spot in the molecule, the bridge between S and CH, is open to attack by different agents, particularly the ions of heavy metals.

The chemist who reads from, or even browses in, this excellent survey of knowledge to the end of the summer of 1946 will see it largely as a series of logical conclusions drawn from these simple facts about the constitution of the penicillin molecule. On the other hand, he will appreciate how much there is yet to be discovered about the exact *modus operandi* of the drug's action on the bacterial cell, about what it is that causes some bacteria to be extremely sensitive to this attack and others quite indifferent and about the small but interesting question of why penicillin K, with its seven-membered straight hydrocarbon side chain, should be apparently more easily destroyed than the other three, for, superficially, examination of the four formulae would point to it as likely to be the stablest.

Although those parts of the penicillin story of most interest to the chemist are rightly treated in a somewhat condensed manner, because this book was primarily designed by the Pharmaceutical Society for its own members, not only can these portions be read by chemists with interest and profit, but the rest of the book also is packed with information that must often be of use both to pharmacists, and to those in the sister professions of medicine and chemistry.

The book is on the whole accurate and clearly written, though the writing bears signs, if not of a dual author, at least of an author who has at some times been in much more of a hurry than at others. A number of minor criticisms could well be made. One can question the desirability of using the word glycerine (*e.g.*, on p. 33) when describing the effects of the chemical substance glycerol; again, in referring to the effect of heavy metals on penicillin, it is not made sufficiently clear that the metals themselves probably have no destructive action, but only their ions in solution. The accuracy of writing, to which I have referred above, is hardly maintained on page 55, where it is said, of a full bacteriological investigation, "it should not be awaited before administration is commenced."

In general, however, it must be agreed that the author (or group of authors) has very successfully dealt with the greatest difficulty that arises over the writing of books like this. They tend to go out of date so rapidly that, even in normal times, a second edition appears to be needed immediately the first has been issued. In present circumstances things are often much worse, though this particular book seems almost to have created a record in the post-war world by the speed of its publication, judging by the information and references that are to be found in a volume bearing the issuing date of October, 1946. All the same we have already to modify some of the statements in the book, both on the experimental and on the manufacturing side. It is, for example, no longer true that the half million unit size of penicillin must consist of material that has been freeze-dried in bulk, for the technical problems involved in drying that amount *in situ* have now been surmounted. Nevertheless, in spite of any advances made since the manuscript of this book went to press, it remains one of the most concise and precise accessible sources of information about one of the most striking and useful drugs ever made available to doctor and patient.

A. L. BACHARACH

GENERAL CHEMISTRY. By E. P. SCHOCH, W. A. FELSING, and G. W. WATT. International Chemical Series. Second Edition. Pp. xiii + 540. London: McGraw-Hill Publishing Co., Ltd. 1946. Price 20s.

This is a first year's text-book of general and theoretical, inorganic and organic chemistry that essays a mean path, in its method of treatment, between the modern and more mathematical outlook and the older descriptive chemistry. In this regard, the authors' prefatory hope "that a satisfactory degree of modernisation has been achieved without sacrifice of an essentially conservative approach," has been realised.

The treatment of the subject matter is superficial, but complete within the range of an introductory course. The sections on the periodic table, atomic structure and nuclear fission are particularly clear. In selecting examples for instructional purposes, the authors have not hestitated to draw upon the most recent advances in theoretical and practical chemistry. Amongst these are to be found the structural formula for *l*-ascorbic acid, a table of vitamins that includes biotin and inositol, a picture of a reverberatory tin smelting furnace erected during the recent war and of the new 60-inch cyclotron at the University of California; also chapters on synthetic rubber, nuclear reactions and artificial radioactive isotopes. Modern sation such as this should help to keep present in a student's mind the ever expanding natu of chemical science and go far to prevent that impression of an already complete and static subject so often created, in the not very distant past, by a similar class of book.

In the more descriptive parts of the book are to be found chapters on metals and alloys, iron and steel, acids, bases and salts; but there is no systematic description of the elements and their compounds.

The text shows some evidence of multiple authorship in its phraseology and orthography; as an instance of the latter, "coworkers" and "overall" (as a variant of total) sometimes carry their meanings more clearly and easily to the mind by appearing in full dress, complete with hyphens, as "co-workers" and "over-all."

With the exception of recognised American variations in spelling and syntax, the writing is, for the most part, in clear closely-knit English; as is but to be expected of a book that carries as its motto a quotation from such a master of scientific prose as Thomas Henry Huxley. It may be added that there is distinct evidence of the authors' having kept this exemplar before them in much of their own writing.

This book is produced in complete conformity with the established standards of its printers and publishers.

F. L. OKELL

SUPPLY OF LABORATORY APPARATUS AND CHEMICALS

THE Council of the Society will be pleased if any member who is experiencing difficulty in obtaining delivery of LABORATORY APPARATUS will send *full details* of such difficulties to the Honorary Secretary, Mr. K. A. Williams, 6, Milner Street, London, S.W.3, in order that *specific instances* may be brought to the notice of the Controlling Authority.

The following communication has been received from the Chemical Council regarding supplies of LABORATORY CHEMICALS—

> Cases of difficulty in obtaining supplies of laboratory chemicals have been brought to the notice of the Chemical Council, who have discussed the question with officials of the Board of Trade. Some of the delays are admittedly due to transport difficulties and shortage of containers, aggravated by the fact that users often do not return bottles, etc., promptly. The Chemical Council has been assured that the Board of Trade will be ready to take up any questions of genuine shortage which are reported with full particulars. Communications should be addressed to R.M.2T, Board of Trade, Raw Materials Department, Horseferry House, Horseferry Road, London, S.W.1.

REPRINTS

THE following cloth-bound reprints of papers read at meetings of the Physical Methods Group, from THE ANALYST, are now obtainable by application to W. HEFFER & SONS LTD., CAMBRIDGE, price 2/6 each.

SYMPOSIUM ON POLAROGRAPHY (from ANALYST, **1946**, **71**, 49–65).

SYMPOSIUM ON CHROMATOGRAPHY (from ANALYST, **1946**, **71**, 251–267).

SYMPOSIUM ON SPECTROSCOPIC ANALYSIS (from ANALYST, **1946**, **71**, 348–376) with Lecture by Dr. H. W. THOMPSON, F.R.S., on "The Use of Infra-red Spectra for Analysis."

THE ANALYTICAL METHODS COMMITTEE REPORT ON THE MICROBIOLOGICAL ASSAY OF RIBOFLAVINE AND NICOTINIC ACID

is now obtainable in the form of paper-covered reprints; price, to members 1/6, to non-members 2/-. Applications, with remittance, should be sent to Mr. J. H. LANE, Editor, THE ANALYST, 7–8, Idol Lane, London, E.C.3.

ADVICE TO AUTHORS

THE Council has approved the following notice by the Publication Committee, which is here given in condensed form.

The Society publishes papers concerned with all aspects of analytical chemistry, inorganic and organic, including chemical, physical, biological, bacteriological and micro methods. Papers on these and allied subjects, by members of the Society or non-members, may be submitted for presentation and publication; they may:

(1) record proposals for new methods and the investigations on which the proposals are based;
(2) record the results of original investigations into known methods or improvements therein;
(3) record analytical results obtained by known methods where these results, by virtue of special circumstances, such as their range or the novelty of the materials examined, make a useful addition to analytical information;
(4) record the application of new apparatus and new devices in analytical technique and the interpretation of results.

Communications.—Papers (which should be sent to the Editor) will normally be submitted to at least one referee, on whose advice the Publication Committee will be guided as to the acceptance or rejection of any communication. Papers or Notes accepted by the Publication Committee may not be published elsewhere except by permission of the Committee.

Abstracts.—The MS. should be accompanied by a brief abstract of about 100 to 150 words indicating the scope and results of the investigation.

Proofs.—Proofs should be carefully checked and returned within 48 hours. Two galley proofs* will normally be sent out, one of which should be retained by the Author.

Reprints.—Ten Reprints are supplied gratis to the Author. Additional reprints may be obtained at cost if the Author orders them directly from the printers, W. Heffer & Sons Ltd., 104, Hills Road, Cambridge, at the time of publication. Details are sent to Authors with the proofs.

Notes on the writing of papers for THE ANALYST

Manuscript.—Papers and Notes should preferably be typewritten.

The title should be descriptive and should set out clearly the scope of the paper. Degrees are now omitted after the names of Authors in the headings of papers.

Conciseness of expression should be aimed at; clarity is facilitated by the adoption of a logical order of presentation, with the insertion of suitable paragraph or sectional headings.

Generally, the best order of presentation is as indicated below:

(*a*) General, including historical, introduction.
(*b*) Statement of object of investigation.
(*c*) Description of methods used. Working details of methods are usually most concisely and clearly given in the imperative mood, and should be given in this form, at least while economy of paper is pressing, *e.g.*, "Dissolve 1 g. in 10 ml. of water and add . . ." Well-known procedures must not be described in detail.
(*d*) Presentation of results.
(*e*) Discussion of results.
(*f*) Conclusions.

followed by a short summary (100 to 250 words) of the whole paper: items (*e*) and (*f*) can often be combined.

Illustrations, diagrams, etc.—The cost of setting up tabular matter is high and columns should therefore be as few as possible. Column headings should be brief or replaced by a number or letter to be used in combination with an explanatory footnote to the table.

Sketches or diagrams should be on white Bristol board, not larger than foolscap size, in Indian ink. Lettering should be in light pencil.

Tables or graphs may be used, but normally not both for the same set of results. Graphs should have the co-ordinate lines clearly drawn in black ink.

References.—References should be numbered serially in the text and collected in that order under "REFERENCES" at the end of the paper. They should be given in the following form:

1. Dunn, J. T., and Bloxam, H. C. L., *J. Soc. Chem. Ind.*, **1933**, **52**, 189T.
2. Allen, A. H., "*Commercial Organic Analysis*," Churchill, London, 1882.

Notes on the Presentation of Papers before Meetings of the Society are appended to the "ADVICE," copies of which may be obtained on application to the Secretary, 7/8, Idol Lane, London, E.C.3

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* During the paper shortage two copies of the MS. will not be insisted on, nor will two galley proofs be sent.

JUNE, 1947 Vol. 72, No. 855

THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

An Ordinary Meeting of the Society was held at 6 p.m. on Wednesday, May 7th, at The Chemical Society's Rooms, Burlington House, London, W.1, with the President, Mr. Lewis Eynon, in the chair. The following paper was presented and discussed: "A Micro-chromatographic Method for the Detection and Approximate Determination of the different Penicillins in a Mixture," by R. R. Goodall, Ph.D., and A. A. Levi, Ph.D., A.R.I.C. The paper was preceded by a brief account of the chemical nature of the Penicillins by F. A. Robinson, M.Sc., LL.B., F.R.I.C.

NEW MEMBERS

Professor Marti Fernando Burriel, D.Chem. (Madrid and Brussels); Albert Cecil Case, B.Sc. (Lond.), A.R.I.C.; Daniel Corkery, M.Sc.; William Francis Jack Cuthbertson, B.Sc., Ph.D. (Lond.), A.R.I.C ; Alfred Davidsohn; Eric Coning Dawson, B.Sc. (Lond.), M.Sc. (Dunelm), A.R.I.C.; Francis Cyril James Poulton.

DEATHS

We deeply regret to record the deaths of

Sir Frederick Gowland Hopkins, Honorary Member.
Robert Ellison.

The Fundamental Laws of Polarography

By J. HEYROVSKÝ

(*Lecture delivered at a Meeting of the Society on October* 31*st*, 1946)

The foundations of the polarographic method are closely connected both theoretically and experimentally with fundamental researches on the significance of electrode potential and polarisation. In the seventies, G. Lippmann introduced the cell consisting of a mercury meniscus in a capillary, as one electrode, and a large mercury pool as the other. The capillary was connected by means of rubber tubing to a mercury reservoir, the height of which could regulate the position of the meniscus in the capillary. The rôle of the large mercury electrode was to ensure that, owing to its small current density, its potential would remain constant, so that the total external E.M.F. applied on the electrodes of this cell would produce changes of potential at the small electrode only. This provided a convenient system comprising a polarisable electrode (the capillary one) and an unpolarisable electrode (the bottom one). From Lippmann's studies of electrocapillarity carried out with this cell Helmholtz was able to work out his theory of electrode potential being the potential of a double-layer condenser, of which one layer lies on the metal and the oppositely charged layer is in the surrounding solution. In 1903, B. Kučera developed Lippmann's method further by raising the mercury reservoir so that mercury would drop slowly out of the capillary. He has shown that the drop-weight method is more convenient for the determination of the interfacial tension (γ) of polarised mercury than Lippmann's method of observations on the meniscus, since the drop-weight, w, is given by $w = 2\pi r\gamma$, r being the radius of the mouth of the capillary. However, w is equal to $m.t$, m being the rate of outflow of mercury from the capillary and t the drop-time; hence, at a constant applied E.M.F., $w = m.t = 2\pi r\gamma = k$ is a constant. Since, however, m is proportional to the height, h, of the mercury reservoir, t must be inversely proportional to h.

Kučera obtained with his method a number of electrocapillary parabolas, some of which, however, showed an anomalous maximum. In 1918 he suggested that the present writer should investigate the cause of such anomalies; in the course of these investigations it became evident that a much better insight into the processes occurring at the dropping electrode could be obtained by measuring the currents which pass through the cell at different voltages. In this respect the dropping mercury electrode was found to be especially suited to show a variety of exactly reproducible phenomena, as it offers the following advantages: (1) it develops always a fresh, regularly renewed surface of mercury and of the adjacent electrolyte solution, so that no time effects due to accumulating products of electrolysis are possible; (2) the large overvoltage on pure mercury prevents hydrogen deposition, so that even alkali metals may be deposited without decomposition of water taking place; (3) since the extent of electrolysis is small, changes in the concentration of the depolariser in solution are negligible, and consequently the measurements may be repeated with the same small amount of solution many times with identical results; (4) owing to the perfectly regular dropping, all processes repeat themselves at each fresh mercury drop with a high degree of exactitude, so that the current is a well-defined function of the dropping-electrode potential.

From the regularity of conditions under which the surface of each drop is developed, Ilkovič, and, later, Rideal and MacGillavry, were able to deduce that the mean current, i_{E}, due to electrolysis of a depolariser present at a concentration C in a concentrated solution of an indifferent electrolyte, is determined by the equation:

$$i_{\text{E}} = 0{\cdot}627\ \nu.\text{F}.\text{D}^{1/2}m^{2/3}t^{1/6}(C-C_0),$$

where ν is the electrovalency, F Faraday's constant, D the diffusion coefficient and C_0 the concentration of the depolariser at the electrode surface, all quantities being expressed in absolute units; i_{E} reaches its limit when $C_0 = 0$, at which condition the depolariser is completely exhausted at the electrode surface. This current is then called "diffusion current"; it gives the "height of the polarographic wave" and is proportional to the concentration C of the depolariser in the body of the solution, so that it serves as a measure of its concentration. From the relationships given above between m, t and h it follows that the "diffusion current" is proportional to $\sqrt{h}$, *i.e.*, it varies as the square root of the height of the mercury reservoir.

The relationship between the current, i, and the potential, π, of the polarised mercury electrode* has been deduced by Ilkovič and the present writer from the formula of Peters

$\cdot \frac{\text{RT}}{\nu\text{F}} \log \frac{[\text{red.}]_0}{[\text{ox.}]_0} . k)$ as

$$\pi = -\frac{\text{RT}}{\nu\text{F}} \log \frac{i}{i_d - i} \sqrt{\frac{\text{D}}{\text{D}'}} . k,$$

where D and D′ denote the diffusion coefficients of the depolariser in its oxidised and reduced states respectively, the electrolytic process being ox. $+ \nu\ominus \rightarrow$ red., *e.g.*, $\text{Pb}^{\cdot\cdot} + 2\ominus \rightarrow \text{Pb}$. The curve, giving this dependence of the current, i, on the potential, π; *i.e.*, the current - voltage curve has the shape of curve 1 in Fig. 1. It has an inflexion for $i = i_d/2$, at which point the concentration of the depolariser at the electrode surface is half exhausted ($C_0 = C/2$) and also the concentration of the lead amalgam reaches half of its final value. The potential, $\pi_{\frac{1}{2}}$, at which this happens is a constant independent of the concentration of the depolariser, C, and of the properties of the capillary (m, t). This is the so-called "half-wave potential" characterising the quality of the depolariser.

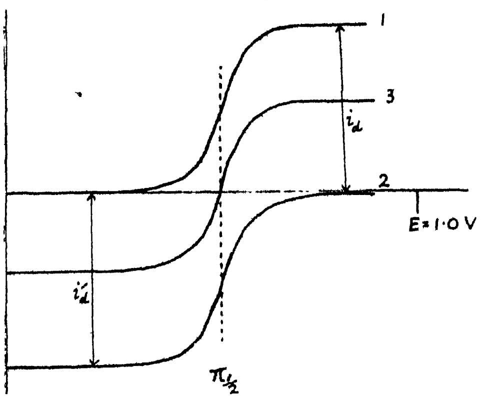

Fig. 1. Current - voltage curves.

If we use instead of pure mercury in the dropping electrode a dilute, say, 0·001 per cent. amalgam of lead and polarise it in a pure solution of an indifferent electrolyte cathodically, starting with a large voltage, say, E = 1·0 v., the current is practically zero (Fig. 1, curve 2). On decreasing the voltage, *i.e.*, imparting to the dropping electrode more positive potentials, we start anodic dissolution of lead, *viz.*, $\text{Pb} \rightarrow \text{Pb}^{\cdot\cdot} + 2\ominus$. At a potential at which half of the lead content from the surface dissolves, the "half-wave potential" of lead is reached and

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* π depends on the voltage V (*i.e.*, external applied E.M.F.) as $\pi = -\text{V} + i.\rho$, where ρ denotes the resistance in the circuit. Since both i and ρ are small, π is practically identical with V.

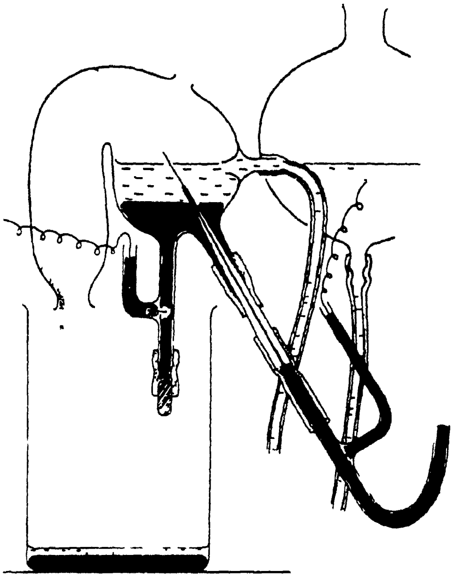

Fig 3 The streaming electrode

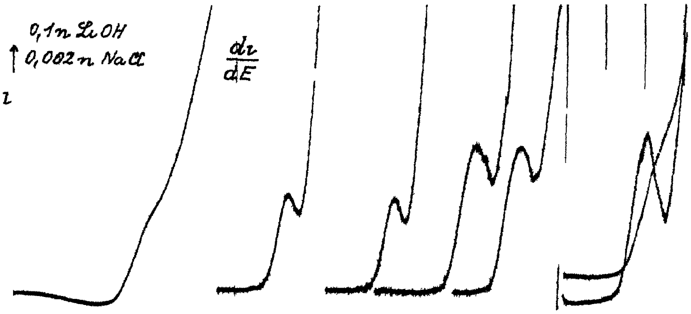

Fig 7 Left the current voltage curve middle its derivative curve, right the current - voltage curve and its derivative

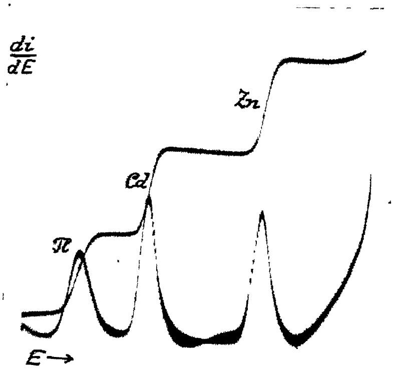

Fig. 8. The current - voltage curve of a solution containing 10^{-3} *N*, $Tl^{\cdot}$, $Cd^{\cdot\cdot}$, and $Zn^{\cdot\cdot}$ and its derivative.

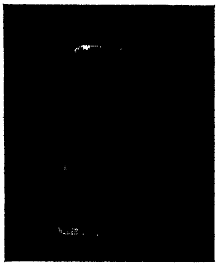

Fig. 10. Oscillographic "spectral line."

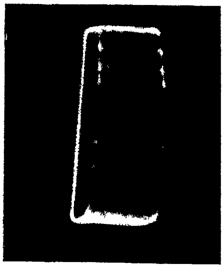

Fig. 11. Oscillographic "spectrum" due to $10^{-3}N$ $Tl^{\cdot}$, $Cd^{\cdot\cdot}$, $Zn^{\cdot\cdot}$, $Fe^{\cdot\cdot}$ and $Mn^{\cdot\cdot}$ in ammoniacal solution.

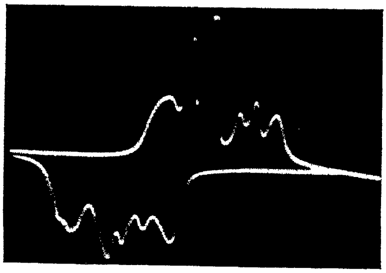

Fig 12. The derivative of the potential - time curve.

when all the lead from the surface of the amalgamated drop is dissolved, an anodic "diffusion current," i'_d, ensues. We thus obtain a curve of the same shape as when using pure mercury dropping into a lead salt in the solution. If the lead amalgam drops into a solution containing a lead salt, there is a cathodic diffusion current, i_d, as well as an anodic diffusion current, i'_d, the first indicating the concentration of lead in the solution, the second that in the mercury. In fact, in polarography we always deal with an electrode equilibrium of the depolariser in its oxidised (ox.) and reduced (red.) states, *i.e.*, with redox potentials. The current - voltage curve for cases in which both the oxidised and the reduced forms are present is represented by the equation

$$\pi = -\frac{\mathrm{RT}}{\nu.\mathrm{F}} \log \frac{i-i'_d}{i_d-i} \sqrt{\frac{\mathrm{D}}{D'}} \cdot k$$

This formula is mostly applied to solutions of redox indicators and other systems containing in the solutions red. and ox. components, *e.g.*, Fe$^{\cdot\cdot}$ — Fe$^{\cdot\cdot\cdot}$, Cr$^{\cdot\cdot}$ — Cr$^{\cdot\cdot\cdot}$, Sn$^{\cdot\cdot}$ — Sn$^{\cdot\cdot\cdot\cdot}$, quinone - hydroquinone, lactoflavin - leuko-base, etc. The study of current - voltage curves gives us, indeed, the same information as the Michaelis "potentiometric titration curves." In these, increasing amounts of a reducing agent, say, Cr$^{\cdot\cdot}$, are added to the depolariser, *e.g.*, Fe$^{\cdot\cdot\cdot}$, whereby the ratio of ox. to red. is changed and the corresponding π is noted (Fig. 2*a*). In polarography the reduction of ox. to red. is effected by the current of electrons, which reduce the depolariser at the interphase, and the corresponding π is shown on the abscissa (Fig. 2*b*). To change diagram *a* into *b* one has first to turn *a* round through 90° and then turn its paper upside down to cover exactly the curve *b*. From this it is evident that all deductions that are derived from Michaelis curves also follow from the polarographic waves, notably as regards the formation of semiquinones and dimers. Moreover, in polarography one of the components suffices, the second being formed at the electrode; this is of special value if one of the forms is unstable. Also the range of negative potentials at which the redox systems may be studied is considerably extended (to —2·0 v. from the 1 *N* KCl calomel zero).

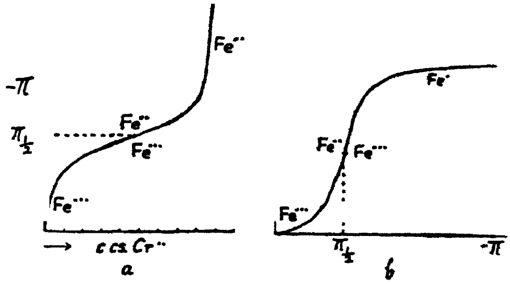

Fig. 2. (*a*) Potentiometric titration curve.
(*b*) Polarographic current - voltage curve.

When examining solutions containing more than one depolariser, we obtain the corresponding number of waves, which allow simultaneous qualitative and quantitative determination of the depolarisers. If the half-wave potentials are near to each other, the waves coalesce so that the determination is uncertain; however, an addition, to the electrolyte, of a compound that makes complexes of different stability with the various depolarisers helps to resolve such coincidences into separated waves. Another difficulty arises when we have to determine accurately small quantities of a baser component in excess of nobler ones, *e.g.*, traces of Zn or Cd in Cu. Then the noblest component (Cu$^{\cdot\cdot}$) forms such a large wave at a positive potential that waves of the minor components, reducible at more negative potentials, are hardly distinguishable at the given sensitivity. This difficulty might be removed by applying a differential method described recently by Semerano and by Kanewsky, who use two solutions in separate vessels with exactly identical capillaries in each; the difference of currents flowing through the two capillaries is registered against the voltage. One of the vessels contains the sample to be analysed, the other only the indifferent electrolyte used in the first solution. Next, the noblest (most electro-positive) component is added to the indifferent electrolyte in such an amount as to make its concentration equal to that in the vessel with the sample, so that the two diffusion currents balance each other. On increasing the voltage the diffusion currents of the baser components may be registered with a large sensitivity. The presence of air does not interfere, since the oxygen waves exactly balance each other. This differential method assumes equal drop-times and synchronous dropping for both electrodes, which is very difficult to maintain. Therefore the present author recommends here the use of two streaming electrodes instead of the dropping ones. One type of the streaming electrode is shown in Fig. 3. It consists of a thick-walled capillary with an inside diameter of 1 to 2 mm., drawn out at the tip to an internal diameter of about 0·1 mm.

It is essential to keep the level of the solution in the vessel constant, so as to have always the same length of the electrode given by the jet of mercury. Two such electrodes, each surrounded by a solution in the same indifferent electrolyte, coupled according to the differential scheme, can be used to show slight differences between samples of similar composition. In Figs. 4 and 5 curves obtained with two streaming electrodes are shown, the solutions being exposed open to the air; the sensitivity of the galvanometer was decreased 1:30. The method is very sensitive, but the consumption of mercury is large, amounting to 600 to 1000 g. per hour (under a pressure of about 50 cm. of Hg). One streaming electrode alone is not suitable for analytical purposes, since the charging (or "capacity," *i.e.*, "condenser") current is about 100 times larger than that of the dropping electrode.

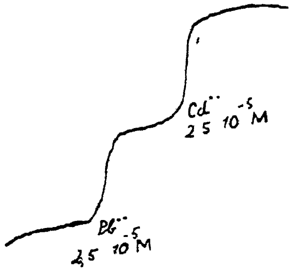

Fig. 4. Comparison of solutions: one solution pure *N* HCl, the other containing $Pb^{\cdot\cdot}$ and $Cd^{\cdot\cdot}$ in a concentration $2{\cdot}5.10^{-5}M$.

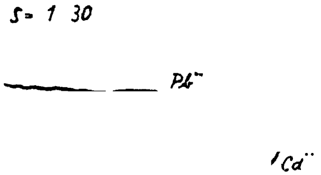

Fig. 5. One solution contains $Pb^{\cdot\cdot}$, the other $Cd^{\cdot\cdot}$ ions in a concentration $2{\cdot}5.10^{-5}M$, both in *N* HCl.

Another method, which enables small quantities of baser constituents to be determined in presence of excess of nobler ones, and which at the same time gives new information on the shape of current - voltage curves, consists in obtaining the "derivative" curve, $\frac{di}{d\pi} - \pi$. Here again a double electrode is used (Fig. 6), but dipping into one solution only. Since synchronous dropping is difficult to attain, the drop-time is reduced (0·8 to 1 sec.) without synchronising the drops. Thus the curves in Figs. 7 and 8 were obtained. The equation of the current - voltage curves gives for the differential quotient at the half-wave potential $\frac{di}{d\pi} = -\frac{\nu.F}{4RT}.i_d = 10\nu.i_d$, as a maximum. The advantage of this method for analytical applications is due to the ease with which the half-wave potential and the height of the maximum, indicating the quantity required, are determined.

The precision with which a component may be determined polarographically reaches in favourable case ±1 per cent. of its absolute amount. Where greater exactness is required "polarometric titrations" may be used. In these the diffusion current of the component is followed at a certain constant voltage as it is diminished during precipitation with a suitable agent. The end-point of the titration is shown by the minimum value of the diffusion current; this minimum is best found graphically from a hand-drawn diagram, two measurements before and two after the end-point being necessary. By this method the precision is increased to 0·1 per cent. The component or the precipitating agent has to act as a depolariser. It is best when both are polarographically active, because in this case a very sharp minimum of the diffusion current is obtained (*e.g.*, nickel with dimethylglyoxime). The school of I. M. Kolthoff has worked out many analytical applications of these polarometric (called also "amperometric") titrations. An interesting case is encountered in the polarometric titration of thorium salts with fluorides, neither of which is a depolariser, yet the presence of $Th^{\cdot\cdot\cdot\cdot}$ ions provokes a wave of nitrate ions added for that purpose. As soon as all thorium ions are precipitated the nitrate wave disappears.

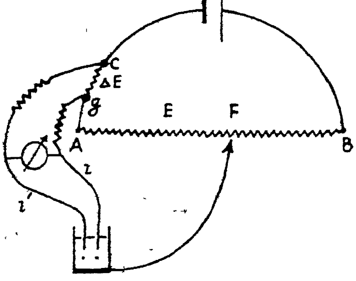

Fig. 6. Scheme for obtaining the derivative curve.

In the last few years a new development of polarographic research has been started through the use of potential - time curves, obtained on the fluorescent screen of the cathode ray oscillograph.[2]

The voltage of the ordinary alternating current supply (of 50 cycles per sec.) acts on the dropping mercury electrode and the changes of its potential are recorded on the oscilloscope. The curve shown with pure indifferent electrolyte has the shape *a* (Fig. 9), and a trace of depolariser changes it into the shape *b* (Fig. 9) with a time-lag at the depolarisation potential.

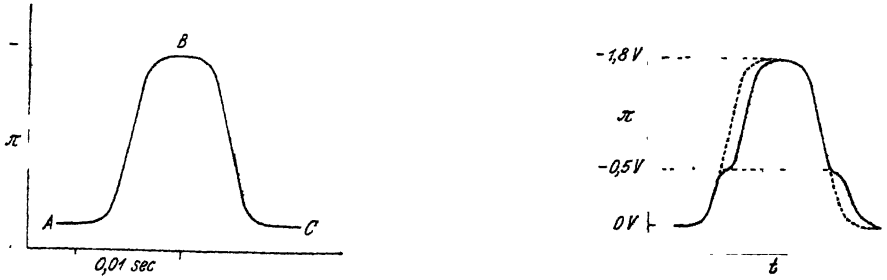

Fig. 9 (*a*). Fig. 9 (*b*).

Oscillographic potential - time curves.

Increased frequency of the time base (to about 100,000 per sec.) changes curve *b* into Fig. 10, where the depolariser is characterised by a line at a certain potential. If more depolarisers are present, the corresponding number of lines appears as an "oscillographic spectrum" (Fig. 11). The quantity of the depolariser is deduced from the "derivative" curve (representing $\frac{dV}{dt} - t$) through the depth of the cut-in, shown on the curve in Fig. 12. To obtain a steady oscillogram the streaming mercury electrode has to be substituted for the dropping electrode.

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PHYSICO-CHEMICAL DEPARTMENT
CHARLES UNIVERSITY
PRAGUE

DISCUSSION

The PRESIDENT, Dr. G. W. MONIER-WILLIAMS, expressed the cordial thanks of the Society to Professor Heyrovský for enabling them to hear this authoritative account of the fundamental principles of polarography from one who was not only the originator but also the leading exponent of the subject. The Society was grateful also to the British Council, to whom they owed this opportunity of welcoming Professor Heyrovský as their guest lecturer.

Dr. J. E. PAGE said it was a great privilege for him to open the discussion on Professor Heyrovský's lecture. Few branches of analysis could have owed so much to one man as polarography owed to Professor Heyrovský. A high percentage of the 1400 papers published on polarography had come from Professor Heyrovský's laboratory and the bulk of the remainder had been directly inspired by work initiated at Prague. He (the speaker) was particularly interested in Professor Heyrovský's recent work with the cathode ray oscillograph and would like to know if he had used this technique to study (i) oxidation - reduction systems such as benzoquinone - hydroquinone and (ii) catalytic steps of the type formed by cysteine and cystine in ammoniacal cobalt buffer solutions.

Mr. J. HASLAM said it was a privilege to have had the opportunity of listening to the lecture of Professor Heyrovský, which was of fundamental value to the analyst. More and more they saw the fundamental nature of the work, which made possible the determination of reducible substances by clean and neat methods, and they saw also the selective nature of the polarographic method, as in the determination of nitrobenzene in aniline, for which the chemical method of reduction with titanous solution only gave a figure for total reducible matter calculated as nitrobenzene. He thought that in the future when they had more information about the correct ground solutions, the polarographic method would be of great value in that difficult problem for all organic chemists—the rapid quantitative determination of small amounts of various metals present in the sulphuric acid digestion products of organic materials.

Mr. F. STEGHART, speaking of the Ilkovič equation, said that experiments by Maas and Kolthoff seemed to confirm its validity, but some experiments in the laboratory with which he was associated threw some doubt on it. In these experiments there was included in the polarographic circuit a high-speed high-precision amplifier designed in such a way that no interference with the current took place. The output from the amplifier was connected to two instruments, one representing the ordinary highly damped galvanometer and the other a high-speed high-precision recorder. Whilst the damped recorder, which for all practical purposes gave an exact replica of the ordinary galvanometer, confirmed the tests by Maas and Kolthoff, the high-speed recorder contradicted them. The curve obtained was practically never in agreement with one-sixth power of the time, but varied usually between two-thirds and one-third power. As the difference between such curves and the curve of one-sixth power is comparatively small and would only be shown by a highly accurate recorder, it appeared rather likely that the previous tests and those carried out by Ilkovič to obtain confirmation of his theory were obtained only because of the inadequacy of the instruments used at the time. The results of the experiments described above do not affect the practical usability of the polarograph in any way, but they make it appear very likely that the Ilkovič equation describes only part of the effect and that other factors, *e.g.*, the turbulence near the surface of the drop, may play a part. Furthermore, it appeared from micro-photographic experiments that the increase of the mass of the drop is not a linear function of the time, and if this is taken into account it increases the discrepancy between the actual record and the one-sixth power law. From all these tests it appeared that further investigation into the basic theory of the polarograph would be of great interest and might lead to further improvement in the usability and accuracy of the instrument.

Professor HEYROVSKÝ, in reply to Dr. Page's question concerning the oscillographic investigations with oxidation - reduction systems such as benzoquinone - hydroquinone, said that this particular case—as well as that of quinhydrone—shows perfect oscillographic reversibility and so does the system cystine - cysteine. Such red-ox electrode reactions must, therefore, come to the thermodynamically defined state of equilibrium with an extremely high rapidity. The catalytic reactions with cysteine were not yet investigated, but the reaction of blood-proteins in ammoniacal cobalt buffer solutions were found to show characteristic time-lags in the oscillographic potential - time curve, again proving the high rate of this catalysed reaction.

To Mr. Haslam's remark on the possibility of the determination of traces of metals in organic materials he expressed the opinion that such determinations, *e.g.*, of iron, copper, bismuth, antimony, lead, thallium, cadmium, zinc, and so on, are possible without difficulties.

In reply to Mr. Steghart, Professor Heyrovský remarks that the validity of the one-sixth power law of Ilkovič has been firmly established experimentally by measurements with a torsion thread galvanometer of 0·01 sec. period of swing (*Collection of Czechoslovak Chemical Communications*, **8**, 1936, p. 31), and also by the fact that the diffusion current, i_d, varies as the square root of the height of the reservoir, h, as was pointed out at the beginning of this paper (*Collection*, **6**, 1934, p. 510). Thus the one-sixth power holds equally for momentary currents and for the mean current. If a one-third power law held, then i_d would be proportional to the cube root of h (for i_d would equal $k.m^{2/3}.t^{1/3} = k'.h^{2/3}.h^{-1/3}$); and if a two-thirds power law held, then i_d would be independent of h (for i_d would equal $k.m^{2/3}.t^{2/3} = k'.h^{2/3}.h^{-2/3}$). Experimentally the two latter cases are found only when a kinetic reaction takes place at the interphase (see *e.g.*, Wiesner, *Collection*, **12**, 1947, p.64), or when motion of the electrolyte occurs (as with maxima, when $i = k.t^{1/3}$). Turbulence was never observed in the outflow of mercury, and as the rate of outflow is constant and given by the height of the mercury reservoir, the mass of the drop must be a linear function of time. There is a small capillary back-pressure at the beginning of the formation of each drop, amounting to a few per cent. of h and disappearing as the drop grows; this small effect, however, cannot account for the discrepancy referred to by Mr. Steghart.

Ilkovič's law was derived on the assumption that each particle of the depolariser, say a cation, is deposited as soon as it touches the electrode. However, oscillographic investigations carried out by the present author (see *e.g.*, Faraday Soc. Discussion on Electrode Reactions, April, 1947), show that the transfer of two or more electrons, *e.g.*, in the deposition of $Cu^{\cdot\cdot}$ or $In^{\cdot\cdot\cdot}$ ions, involves consecutive (dismutation) reactions; in such instances—encountered in sulphate or nitrite solutions—the electro-deposition is retarded and the assumption of Ilkovič does not hold; consequently, also the one-sixth power relationship changes towards the one-third power. But such cases are anomalous. As soon as excess of chloride is added, the law of Ilkovič holds, just as it holds in the electro-deposition of any univalent cations ($Tl^{\cdot}$, $Na^{\cdot}$, $K^{\cdot}$) in any solution.

The Determination of Acetyl Groups by Trans-Esterification

By R. G. STUART

THE method of assay of acetyl groups by catalysed trans-esterification to ethyl acetate was investigated by Matchett and Levine.[1] They used absolute alcohol as a solvent, concentrated hydrochloric acid as a catalyst, fractionation through a column packed with glass helices, and intermittent small-volume take-offs into a receiver charged with excess of alcoholic alkali. The take-off device resembled that shown at E in Figure 1. The efficiency of the method depended on the difference in boiling-points between the ternary mixture, water, alcohol and ester, and the binary, alcohol and water. O-Acetyl compounds were given about two hours fractionation, from 2 ml. of acid in 50 ml. of alcohol, with a 2-ml. take-off every 15 min. N-Acetyl compounds, reacting more slowly, received four hours treatment, with 3 ml. of catalyst and similar take-offs, at half-hour intervals.

In these laboratories, the first attempt to use this method was made on N-acetylphenetidine, which is used as an "analytical standard" for acetyl groups. For quantitative recovery, it was found necessary to distil a large volume of alcohol (more than 100 ml.) and in doing so to refill the still-pot several times. The subsequent titrations had an unduly large possible error, and the time of distillation exceeded 8 hours.

To reduce inaccuracy and running costs, an apparatus was designed (Fig. 1) to allow the redistillation of the alcohol from the receiver into the still-head at the same rate as that from the still-pot into the receiver. It was then possible to examine rates of recovery of ethyl acetate under standardised conditions, the volume in the two flasks remaining constant. At this stage it was found that introduction of a few per cent. of water or of methyl alcohol caused no significant change in rates of recovery, but anhydrous ethanol was retained as a reagent because of greater ease of purification.

APPARATUS (Fig. 1)

The still-pot A (100-ml. round-bottomed pyrex flask, B24 neck, with lugs) is attached by springs to the fractionating column C, which has an effective length of 90 cm., an effective bore of 14 mm., a vacuum jacket (pressure about 1 micron Hg), a working hold-up of 7 ml., a static hold-up of 3 ml., a through-put, just before flooding, of about 20 ml. per minute, and a filling of 4 mm. of Fenske single-turn glass helices.[2,3]

The still-head includes a total reflux condenser D into the U-tube E. This U-tube has a variable constant-ratio take-off through tap F into a visual drip-feed into condenser R, which cools the take-off and refluxes the reagent alcoholic potash in flask B (a replica of flask A). The tube between condenser R and flask B is connected by a 10-mm. bore vertical lagged air tube to a small reservoir P, above tap G, which controls a visual drip-feed into the top of condenser D. Condenser H is a total reflux into reservoir P, and condenser K cools the pressure outlet to flask A and the fractionating column.

The two outlets to the whole system, the tops of condensers H and K, are capped by soda-lime tubes L, attached *via* B10 joints.

For strength, the four condensers are jointed by 10-mm. bore water-carrying tubes, the whole still-head assembly being of glass blown in one piece, with its centre of gravity near enough to that of the fractionating column to allow it to be rested in and supported by the B34 joint which tops the column. The column is clamped at two symmetrical points to the bench framework, and the stillhead is lightly clamped round the jacket of the air-tube at X.

Flasks A and B are heated by small electric heaters, each of about 500 watts, and the upper halves of the glass joints are lightly greased with petroleum jelly.

METHOD OF OPERATION

MACRO ANALYSIS

REAGENTS—

(1) *Anhydrous ethanol* 99·5 *per cent.*—This is freed from aldehyde by distillation in vacuum from 1 per cent. of sodium metal and subsequent fractionation through column C (Fig. 1), using 250-ml. flasks and charging the still-pot with 200 ml. The first 50 ml. contain all the remaining aldehyde bodies, and the bulked first fractions are re-fractionated, separating

the first 50 ml. The bulked main fractions, 140 ml. each, are stored in rubber-stoppered amber bottles.

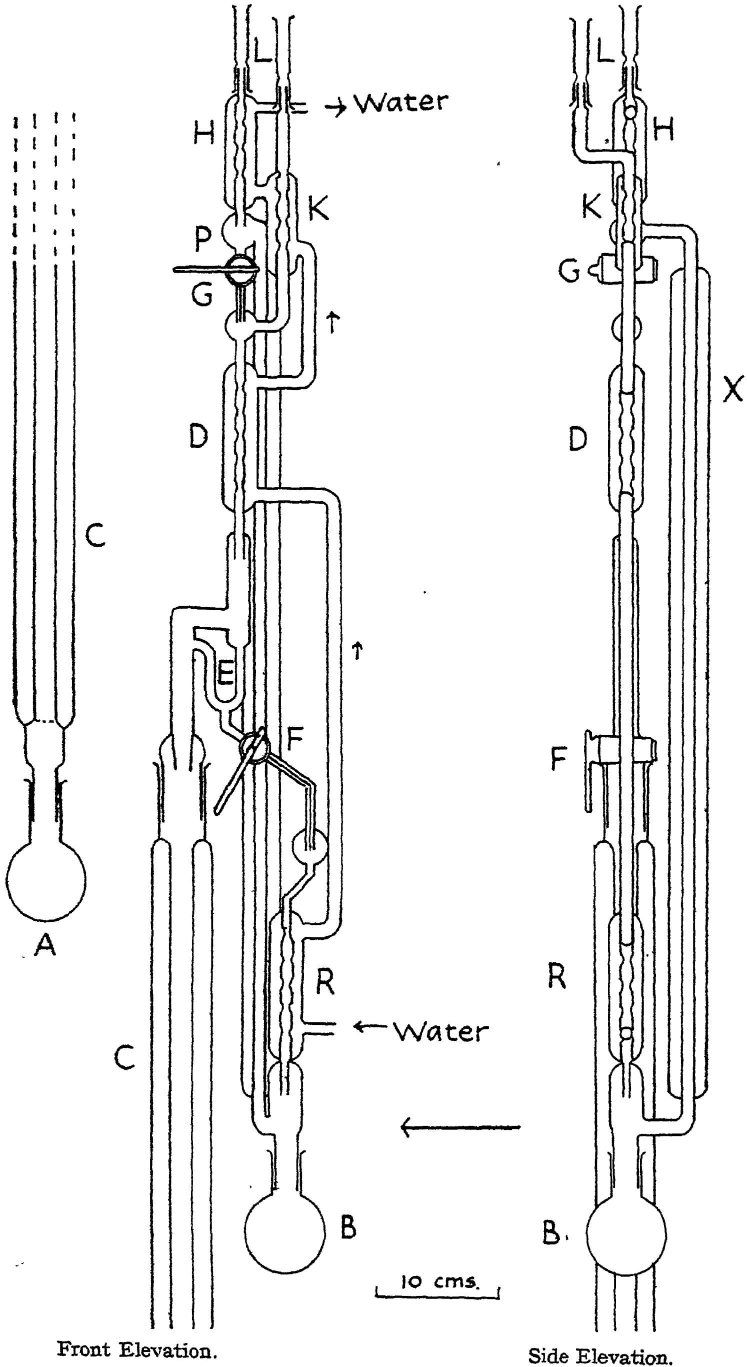


Fig. 1.

(2) *Potassium hydroxide, 0·8 per cent. solution in the purified alcohol*—This solution, which is about 0·11 N, is colourless and remains so indefinitely when exposed to daylight. It is stored in a rubber-stoppered white glass bottle, allowed to stand two days for carbonate

to precipitate and settle, and then pipetted as required from the supernatant clear layer. The temperature correction is about 0·1 per cent. per degree, and the titre changes very slowly, a new standardisation being required monthly.

(3) *Hydrochloric acid, concentrated AnalaR*—35 per cent. of HCl in water.

PROCEDURE—

Charge flask A with 45 ml. of the alcohol, 3 ml. of concentrated hydrochloric acid, a few carborundum chips, and enough of the sample under test to yield 0·043 to 0·086 g. of CH_3CO, *i.e.*, requiring 10 to 20 ml. of 0·1 *N* potassium hydroxide. Charge flask B with 20 ml. of 0·1 *N* potassium hydroxide and 15 ml. of alcohol.

Boil the contents of both A and B until U-tube E and reservoir P are filled with alcohol, and adjust both drip-feeds to about one drop per second. The relative drop sizes of the two feeds have been previously found by experiment to have the ratio 6 : 5, and the rates can be adjusted sufficiently accurately by eye to keep the volumes in the flasks constant, that is, about 35 ml. in each.

Distil from both flasks for one hour from the initial time of boiling of flask A, turn off the taps, and titrate the contents of flask B with 0·1 *N* sulphuric acid, using phenolphthalein as indicator. Titrate a "blank" on the 0·1 *N* potassium hydroxide alone. Deduct 0·15 ml. from the net reading to allow for carbon dioxide in the alcohol (0·1 ml.), and for alkali absorbed by the glass of flask B (0·05 ml.). Carborundum chips are not used in flask B because they are susceptible to attack by alkali. The carbon dioxide in the system becomes concentrated in flask B, and the correction is found from a blank titration on a portion of the alcohol, which does not change in this respect on storage.

It is unnecessary to boil flask B for longer than one hour, because, when the rate of reaction has been found from the first hour's run, the time required to reach a quantitative yield is read off graphically (see Fig. 3 and p. 238); flask B is charged with excess of 0·1 *N* potassium hydroxide, plus alcohol to make up to 35 ml., and distillation from it is resumed for an hour after flask A has been boiling for an hour less than the total time.

For the final back-titration the correction is 0·05 ml. of 0·1 *N* potassium hydroxide.

It is unnecessary to clean the apparatus internally, and it is mounted semi-permanently. No hydrochloric acid remains in the column after draining, and if necessary alcohol can be removed from the column by aspirating a stream of air downwards through it after removal of the soda-lime tubes. (This is sometimes advisable, to remove stale alcohol before an assay.)

With known O-acetyl compounds, one distillation, over a time of two hours, is sufficient.

RESULTS—

To check the efficiency of fractionation, ethyl acetate itself was distilled in the apparatus, with the quantities of reagents as specified, 92 per cent. was recovered in 30 minutes, and 99·5 per cent. in the first hour. From sodium acetate the equivalent of 99·8 per cent. was recovered, as ethyl acetate, in one hour.

For many O-acetyl compounds and N-acetyl compounds curves were drawn of the rates of acetyl recovery (Fig. 2). For all O-acetyl bodies, 99·9 per cent. was recovered in the first two hours; but for N-acetyl compounds the rate varied considerably with the molecular structure, although it was remarkably constant for each compound.

Compound	*% Recovered*
(1) Ethyl acetate	30 min., 92·6; 1 hr., 99·5.
(2) Acetylsalicylic acid	1 hr., 98·0; 2 hr., 99·9.
(3) Acetanilide	1 hr., 59·5; 2 hr., 82·9; 3 hr., 93·1; 7 hr., 99·8.
(4) N-Acetylphenetidine	1 hr., 40·5, 2 hr., 65·0; 5 hr., 93·2; 12 hr., 99·9.
(5) Toluene-azo-diacetylaminotoluene, 2:1:1:4:3 ..	1 hr., 79·2; 3·5 hr., 97·8; 12 hr., 100·2.
(6) N-Methylacetanilide	1 hr., 10·4; 4 hr., 33·2; 8 hr., 59·1.
(7) N-Ethylacetanilide	2 hr., 9·6; 9 hr., 33·3.
(8) Diacetyl-*p*-phenylene-diamine	1 hr., 70·6; 2 hr., 91·4; 3 hr., 97·5; 5 hr., 99·8.
(9) Triacetoxybenzene, 1:2:3 ..	2 hr., 100·0.
(10) Triacetoxybenzene, 1:2:4 ..	2 hr., 100·1.

Other O-acetyl compounds tested included acetic esters of higher alcohols, phenols and sugars; without exception, all the ethyl acetate was recovered in two hours. The same was true with crystalline vinyl acetate, with 98 per cent. of the theoretical amount in the unpolymerised body; further distillation gave no yield.

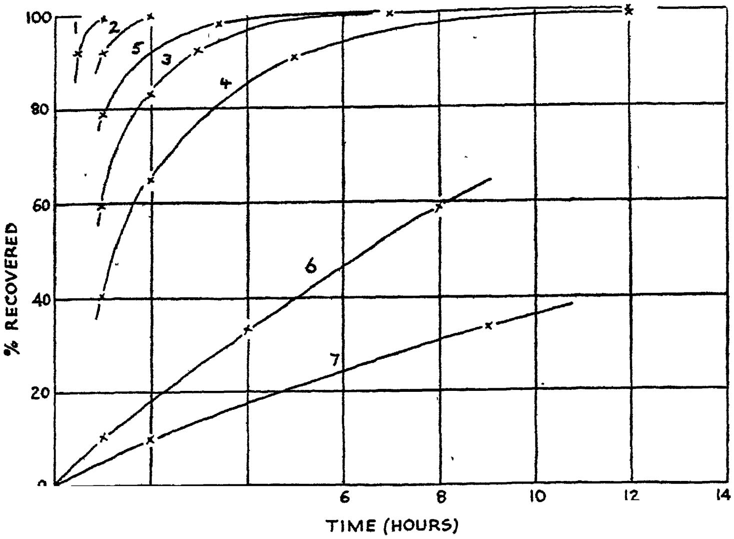


Fig. 2. Rates of Recovery of Ethyl Acetate from Acetyl Compounds.

(1) Ethyl acetate
(2) Acetylsalicylic acid
(3) Acetanilide
(4) N-Acetylphenetidine
(5) 2:1:1:4:3-Toluene-azo-diacetylaminotoluene
(6) N-Methyl-acetanilide
(7) N-Ethylacetanilide

MATHEMATICAL INTERPRETATION—

Every recovery curve obtained so far, including the fractionation curve for ethyl acetate, has been asymptotic and exponential in form; that is, the rate of recovery depends on the concentration of unchanged acetyl body, and the reactions follow the unimolecular law. From this it follows that the shape of each curve should be invariable under constant conditions, and *independent of the weight of acetyl body.* Application of the method to the micro scale, with the same apparatus, showed this to be true.

Exponential form—If the fraction of the original sample that remains unaltered in the still-pot after unit time is a, then after x units of time the fraction, y, remaining will be a^x. Hence, $\log y = x \log a$, and, as for each compound a is a constant, $\log y$ is proportional to x.

Hence a curve in which $\log y$ is plotted against time should be a straight line. The typical constant for each compound is the fraction remaining after unit time.

In practice, the percentage *recovered* is plotted against time, using a log scale, inverted, to measure percentages as ordinates, and a linear scale to measure times as abscissae. The first log cycle is calibrated up to 90 per cent., the second up to 99 per cent., and the third up to 99·9 per cent. (see Fig. 3).

One point in the curve is plotted, and a straight line, drawn through it and the origin, will cut the 99·9 per cent. line at the time required to reach that figure in the trans-esterification. (For convenience, a sheet of graph paper is mounted on a stiff backing, with a cord attached at the zero point.) This procedure is of course only necessary with N-acetyl derivatives.

ANALYSIS OF "UNKNOWN" SAMPLES—

Method—Take burette reading after two equal units of time.

Let a = titration after unit time
b = total titration after 2 units of time.

From these two values the titration, T, corresponding to the completion of the reaction, can be calculated from the formula $\mathrm{T} = \mathrm{a}^2/(2\mathrm{a} - \mathrm{b})$, which is easily derivable from the relations given above.

The percentage recovery in the first unit of time is $\dfrac{100\mathrm{a}}{\mathrm{T}} = \left(2 - \dfrac{\mathrm{b}}{\mathrm{a}}\right)100.$

From this percentage, the time required to reach, say, 99·9 per cent. is read off on the graph, and the distillation completed.

These methods have been used in the analysis of research intermediates, and the rate of reaction can be used to suggest the location of the acetyl group, although the possibility of more than one type being present must be borne in mind.

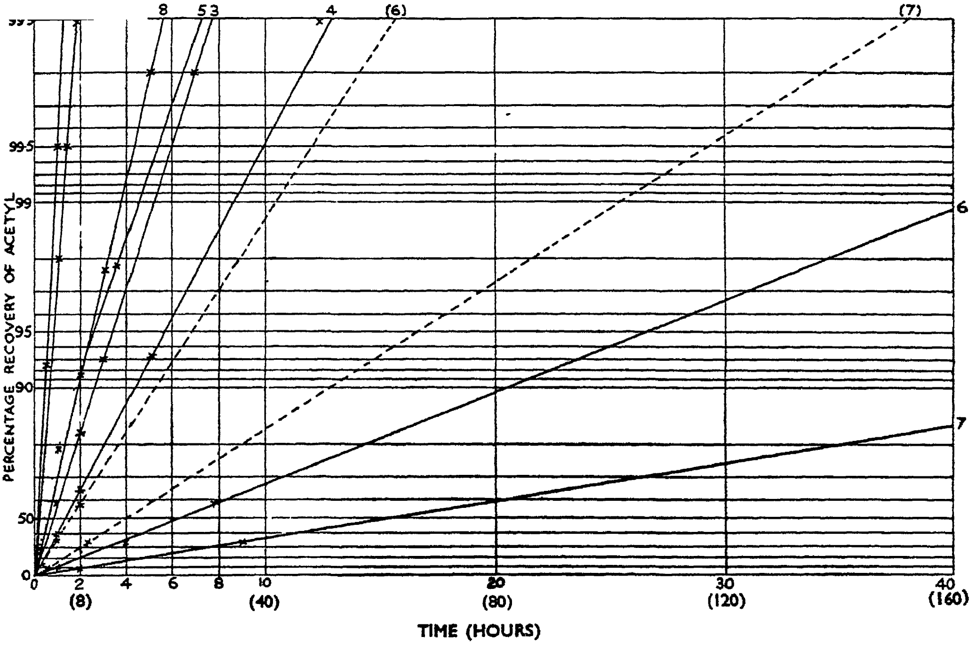


Fig. 3. Rates of Recovery of Ethyl Acetate from Acetyl Compounds.

(1)	Ethyl acetate	(5)	2:1:1:4:3-Toluene-azo-diacetylaminotoluene
(2)	Acetylsalicylic acid	(6)	N-Methylacetanilide
(3)	Acetanilide	(7)	N-Ethylacetanilide
(4)	N-Acetylphenetidine	(8)	Diacetyl-*p*-phenylenediamine

N.B.—Dotted lines (6) and (7) indicate a reduced time-scale for 6 and 7; the corresponding time figures are given in parenthesis at the bottom of the graph.

REVERSIBILITY OF REACTION (on cooling)—

The necessity of sometimes allowing the still-pot to stand overnight, and to continue its heating next day, made advisable a check on the effect of this. Using acetanilide, refluxing two hours, standing overnight and then distilling one hour, 93·5 per cent. was recovered. From the exponential curve for this compound (measured in one day, without cooling) the theoretical recovery is 93 per cent. in 3 hours. It follows that the reversibility of the reaction under these conditions is not significant and that probably a small amount of trans-esterification occurs during cooling and re-warming. The rapid removal of ethyl acetate from the reaction chamber into the U-tube will reduce the reverse reaction, if any occurs. O-Acetyl compounds were not considered.

EFFECT OF CONCENTRATION OF CATALYST—

Following are results obtained with acetanilide and acetylsalicylic acid when different amounts of hydrochloric acid were used.

(1) Acetanilide: 1 hour's distillation and trans-esterification.

1·5 ml. of hydrochloric acid;	45 ml. of alcohol	acetyl recovery	38·5 per cent.
3·0	45	„ „	59·5 „ „
6·0 „ „ „ „	45 „ „ „	„ „	75·0 „ „

(2) Acetyl-salicylic acid: 1 hour.

1·5 ml. of hydrochloric acid	45 ml. of alcohol	acetyl recovery	96·8 per cent.
3·0 „ „ „ „	45 „ „ „	„ „	99·0 „ „
3·0 „ „ „ „	45 „ „ „	„ „	97·8 „ „
6·0 „ „ „ „	45 „ „ „	„ „	98·8 „ „

From these figures it appears that an O-acetyl trans-esterification is not significantly affected by alteration of the concentration of hydrochloric acid, which appears to act as a true catalyst. With N-acetyl compounds, the rate plotted against the concentration of acid appears to follow a curve similar in shape to the trans-esterification curves. The application of this to the speeding of reactions is limited by the capacity of the column to retain hydrochloric acid vapour under the working conditions. When 3 ml. of concentrated hydrochloric acid were used, the quantity of chloride in the distillate was not significant, even when, on one occasion, flask A was accidentally allowed to run dry; but with 6 ml. of the acid its rate of distillation was equivalent to 0·15 ml. of 0·1 *N* per hour. The column could be lengthened to increase retention.

For convenience, the volume of hydrochloric acid used in all determinations was kept at 3 ml.

MICRO ANALYSIS

METHOD—

The distillation rates are left unchanged, but the total volume of alcohol used is reduced from 80 ml. to 60 ml. In flask A are 35 ml. of alcohol and 3 ml. of concentrated hydrochloric acid, and in flask B, 20 ml. of 0·01 *N* alcoholic potassium hydroxide plus 5 ml. of alcohol. Enough sample is weighed to give a net titration of less than 10 ml., the back titration being carried out with 0·01 *N* sulphuric acid, and neutralised phenolphthalein as indicator. As before the only significant errors are due to carbon dioxide in the alcohol (usually ≡ 0·4 ml. of 0·01 *N* per 20 ml.), and to the effect of boiling 0·01 *N* alkali in a glass flask (usually ≡ 0·2 ml. of 0·01 *N* per 20 ml. per hour). In practice, only a blank titration on 20 ml. of alcohol is necessary. There is less margin of error for variations in rate of distillation and re-distillation; but the rate of trans-esterification of N-acetyl compounds is increased about 10 per cent. The relatively larger excess of 0·01 *N* potassium hydroxide in flask B is to ensure that hydrolysis of the distilled ethyl acetate will be rapid enough to prevent its remaining in circulation.

RESULTS—

With known compounds the curves obtained were, as expected, similar to those obtained on the macro scale, with an approximately 10 per cent. increase in the rates of reaction of N-acetyl compounds. The method has been applied in the analysis of a number of research intermediates, both for percentages and for identification.

DISCUSSION

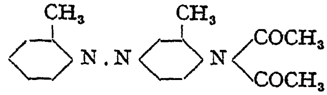
A trans-esterification under these conditions is essentially a hydrolysis of the acetyl compound, followed by an esterification of the acetic acid. This esterification is in all cases at a uniform rate, not significantly affected by the concentration of catalyst. The wide differences in the rates of recovery depend solely on the rates of initial hydrolysis. This rate is fast and uniform throughout a wide range of O-acetyl compounds, and is of the same order as the rate of esterification, because the two together give a rate of recovery not far below that for ethyl acetate. (The exponential form of the straight distillation is due to the mechanism of fractionation, including the slight washing-out effect of the distillate from flask B crossing the inflowing distillate from flask A).

It follows that for O-acetyl compounds the reaction at the boiling-point is almost instantaneous and ionic and depends on the hydrogen ion concentration, which itself has a maximum not affected by a large excess of strong acid.

With N-acetyl compounds, the rate of hydrolysis depends on the concentration of hydrogen chloride, in accordance with Dawson's "Dual Theory of Acid Catalysts," which ascribes the catalytic effect to the concentration of HCl molecules, in addition to that of H and Cl ions. This leaves a wide field of investigation into other causes of variation, namely, the shape of the molecule and the effect of substitution, especially of the spare H atom of the acetylamino group. A recent experiment points to the molecular shape as the prime influence; because N-acetyl-diphenylamine reacts more rapidly than methyl acetanilide, and this more rapidly than the ethyl derivative.

The difficulty of dislodging the strongly held N-acetyl by collision with hydrochloric acid molecules or ions is increased by a protective effect of substituted groups and by the subsequent decrease in polarity, which probably more than counterbalances the decreased strength of the bond holding the acetyl group. Once the dislodging has occurred, the acetyl group will be esterified, the chances of the necessary collision being very high, for there are initially, with 0·1 N solution, about twenty hydrochloric acid molecules to every acetyl. Also the freed amino group will be much more likely to attach a hydrochloric acid molecule than a free acetyl group. With the large excess of hydrochloric acid molecules, the collision rate will depend on the concentration of acetyl compound only; hence the first-order reaction, characterised by an exponential curve.

An interesting case is that of 2:1.1:4:3-toluene-azo-diacetylaminotoluene—



This compound behaves as would a mixture of an O-acetyl compound yielding 100 per cent. of the theoretical amount of acetyl in two hours, and an N-acetyl compound yielding 58 per cent. in one hour. A mixture of this kind would give 79 per cent. of the total acetyl in one hour, 96 per cent. in three hours, and 99·8 per cent. in six hours, figures which agree with the curve obtained. It would be expected that one group would be released, or expelled, at the same rate, approximately, as an O-acetyl group, and that the remaining group would be more strongly held. The derived figure of 58 per cent. per hour for the mono-acetyl derivative is similar to that for acetanilide, faster than that for acetyl-phenetidine (40·5 per cent.) and slower than that for diacetyl-*p*-phenylenediamine (70·6 per cent.).

Every trans-esterification of an N-acetyl compound so far investigated has followed a fixed unalterable rate with remarkable accuracy, so much so that any variation from the theoretical curve showed an error in the technique, and thus helped in the improvement of design and method.

SUMMARY—

Determination of acetyl groups led to the investigation of the rate of trans-esterification of acetyl compounds to ethyl acetate, with hydrochloric acid as catalyst, and to the design of an apparatus which improved the accuracy of the method. All trans-esterifications followed exponential curves, the shape of the curve depending on the molecular structure of the acetyl compound. The time required to reach sufficiently quantitative yields was arrived at graphically. O-acetyl compounds yielded 100 per cent. after two hours. N-acetyl compounds gave rates depending on the substitution of the N hydrogen atom, on the substituent groups in other parts of the molecule and on the concentration of hydrochloric acid.

My thanks are due to Mr. I. J. Fine for the glass-blowing construction, to Mr. T. Tusting Cocking for his advice and encouragement in the experiments described and to the Directors of The British Drug Houses, Ltd., for their permission to publish the results.

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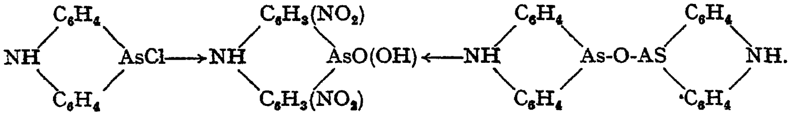
The Determination of Micro-Quantities of some Derivatives of Phenarsazine

BY H. BARNES

THE usual method for the determination of derivatives of phenarsazine involves wet oxidation of the compound followed by determination of the contained arsenic (Sartori[6]). When only small quantities of the arsenical are available either the Gutzeit method or the determination of the arsenic (after reduction and volatilisation as trichloride or as arsine) by the ammonium molybdate reagent, could no doubt be used. Delga[1] has described a colour reaction of phenarsazine chloride; a yellow colour is obtained when aqueous solutions of this substance, in concentrations of 8 mg. or more per litre, are treated with a reagent consisting of silver nitrate in glacial acetic acid.

This paper describes a simple, sensitive, although not specific method for determining some phenarsazine derivatives. Up to the present it has been principally used with pure solutions of the arsenicals in organic solvents (see, however, p. 243), but it has proved of considerable value in toxicity work. It depends upon the action of concentrated nitric acid on the arsenical and the subsequent addition of sodium hydroxide, whereupon an intensely red coloured solution is obtained.

The nitro derivatives of 10-chloro-5 : 10-dihydrophenarsazine and the corresponding phenarsazinic acids yield red salts with sodium hydroxide when the nitro groups are in the 2 and 8 positions (Gibson and Johnson,[2] Raiziss and Gavron,[5] Wieland and Rheinheimer[8]). Further, Schmidt[7] has shown that hot nitric acid oxidises and nitrates 10:10′-oxy-5:10-dihydrophenarsazine. It is suggested, therefore, that under the conditions used in the method given below, both oxidation and nitration take place with the production of the 2:8-dinitro derivative of the phenarsazinic acid, which on addition of sodium hydroxide yields the intensely red coloured quinonoid aci-salt.



METHOD

Reagents—

Nitric acid (A.R.), concentrated.
Aqueous acetone, 50 per cent. v/v solution.
Sodium hydroxide, (A.R.), 10 per cent. aqueous solution.
Sodium potassium tartrate (A.R.), 10 per cent. aqueous solution.

Procedure—

Transfer the test solution to an evaporating dish and remove the solvent on a hot water bath. Add 2 ml. of nitric acid and evaporate just to dryness on a vigorously boiling water-bath. After cooling, dissolve the residue in 10 ml. of aqueous acetone with the aid of a rubber-tipped glass rod and add 1 ml. of the tartrate solution. Add 0·4 ml. of sodium hydroxide solution with stirring. A red colour is produced on the addition of the alkali. Transfer the solution to the absorptiometer cell and measure the transmittance. Calibration curves may be obtained by the use of benzene solutions of phenarsazine derivatives. A blank should be run on the reagents and solvents used.

Measurement of transmittance—

For the determination of transmittances the Spekker photo-electric absorptiometer has been employed throughout, with a 1-cm. cell and green filters (Ilford 604). The instrument is set at 0·400 against distilled water. It has been the practice first to set the instrument and take a reading of the unknown; the instrument is then reset and, with the shutter closed, the drum is moved into the previous position of balance. The unknown is then placed in position and the final adjustment made rapidly.

DISCUSSION

1. The method has been applied to phenarsazine chloride (5-chloro-5:10-dihydrophenarsazine), phenarsazine oxide (10:10′-oxy-5:10-dihydrophenarsazine) and 5-ethyl-5:10-dihydrophenarsazine. Preliminary tests have indicated that the method could be applied to other derivatives of phenarsazine and to compounds with a similar structure.

2. The maximum absorption is obtained with use of green filters (Ilford 604). With a 1-cm. cell the calibration curves are linear over the range 5 to 25 μg. for all the above substances.

3. The limit of the method as given is of the order of 2 μg. of arsenical. It should be possible to determine smaller quantities by reducing the volume of the final solution, but this would necessitate the use of a smaller cell than the standard 1 cm. Spekker cell (approximate volume 10 ml.) and slight changes in the technique may then be found necessary.

4. The spread of the calibration curve indicates that differences of 1 μg. correspond approximately to a drum difference reading of 0·010 (*i.e.*, one drum division).

5. The tartrate solution prevents the precipitation, when the solution is made alkaline, of any small quantities of impurities carried over from the reagents.

6. Care should be taken to bring all the material into solution when the aqueous acetone and the alkali are added; the upper portions of the dish should be carefully rubbed down since the substances show a tendency to "creep."

7. The transmittances should be determined within a short time; the red colour fades, particularly if the solution is left in the evaporating dish. In a covered cell no significant change takes place within 10 minutes.

8. Table I gives a series of results obtained by the method.

TABLE I

AMOUNTS IN MICROGRAMS

Phenarsazine chloride		5-Ethyl derivative		Phenarsazine oxide	
Present	Found	Present	Found	Present	Found
3·4	4·0	16·4	17·2	16·8	16·8
5·0	5·8	—	—	—	—
4·4	4·0	25·2	25·6	15·6	16·2
6·2	5·4	29·6	30·4	20·0	19·4
11·8	11·4	25·6	25·6	23·2	22·6
12·2	12·2	20·0	19·0	26·4	25·2
12·8	13·4	13·2	12·0	17·6	16·8
14·0	13·6	10·4	10·6	28·4	27·2
14·8	14·8	—	—	15·6	15·0
15·0	14·8	—	—	—	—
15·2	15·0	—	—	—	—
15·2	15·4	—	—	—	—
15·2	15·2	—	—	—	—
25·0	24·0	—	—	—	—
28·0	27·6	—	—	—	—

THE DETERMINATION OF PHENARSAZINE CHLORIDE IN AQUEOUS SOLUTION

Derivatives of phenarsazine have been used in experimental anti-fouling compositions[3] and assessments of raft exposures have indicated their effectiveness. In recent work on the behaviour of anti-fouling compositions containing cuprous oxide as the poisonous pigment, a so-called leaching technique has been developed (Harris,[3] Ketchum *et alia*[4]). Painted panels (3″ × 1″ microscope slides, 12·9 sq.cm. painted area) are exposed in the sea and at regular intervals the rate of loss of cuprous oxide (leaching rate) is measured by a standardised laboratory method. These tests of leaching rate have proved to be of great value in interpreting the behaviour of anti-fouling compositions and the technique has been adapted to investigate the rate of release of phenarsazine derivatives from compositions containing them, with or without cuprous oxide. In a four-hours leaching test, using 60 ml. of sea water, a "normal" composition will lose up to 30 μg. of arsenical. A relatively simple method was required, so that large numbers of test panels could be examined, and an application of the method outlined above appeared possible. Preliminary investigations indicated that under the prescribed conditions, in a slightly alkaline medium, hydrolysis of small amounts of phenarsazine chloride was virtually complete and the following method has been used for sea water solutions of this substance; in view of the rather specialised application only a brief description is given. The method must be regarded as of a preliminary nature, since the work has been interrupted before a more complete investigation was possible.*

The sea water extract from the leaching test (60 ml.) is transferred to a 250 ml. separating funnel and shaken for three minutes with an equal volume of chloroform; after the two layers have separated the chloroform layer is run off into a small distilling flask and the major portion of the solvent removed on the water bath. The remaining solution (about 3 ml.) is transferred to an evaporating dish, the flask is rinsed twice with small amounts of chloroform and the washings are added to the bulk. The solvent is then completely removed on a hot water bath and the residue treated exactly as in the direct procedure already described. (Under certain circumstances it may be necessary to centrifuge the solution after development of the red colour). A calibration curve is constructed using sea water solutions of phenarsazine chloride which have been standing for some hours. In order to obtain satisfactory results it is essential to adhere to standardised conditions. Blanks are run on extracts from panels painted with a composition in which the arsenical is replaced by an inert pigment.

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* A progressive hydrolysis on shaking a benzene solution of phenarsazinechloride with alkaline solutions has been demonstrated by analysis of the benzene layer after varying periods. This hydrolysis is suppressed on replacing the slightly alkaline medium by concentrated hydrochloric acid. Preliminary work indicates that as with most substituted derivatives of arsenic trichloride, phenarsazine chloride is comparatively readily hydrolysed—the hydrolysis being difficult to detect or determine owing to the extreme insolubility of the product in water.

The distribution of the hydrolysis product is in favour of the water and this, together with the more involved procedure, renders the results less accurate than those of the direct method. Nevertheless, several hundred determinations by the method have indicated that it is capable of yielding useful information on the behaviour of phenarsazine chloride in anti-fouling paints.

The author is indebted to the Marine Corrosion Sub-Committee of the Iron and Steel Institute for permission to publish this work and to Professor J. E. Harris for his interest in it.

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February, 1947

The Separation of Crystals and "Gums" on the Micro or Semimicro Scale

BY A. L. BACHARACH

(*Communicated to the Annual General Meeting of the Microchemistry Group on January* 31*st*, 1947)

IT often happens during work on the isolation of individual compounds from complex materials of biological origin—and also, doubtless, during preparative and synthetic operations—that a rather intractable mixture is obtained, consisting of a relatively small quantity of obviously crystalline nature embedded in a more or less viscous fluid. Recrystallisation of such mixtures, with a view to isolating the crystalline constituent, is at best fraught with appreciable losses; at worst, it results in the whole of the mixture being taken up in the solvent and ultimate recovery, by evaporation, of the fluid material holding the crystals no longer in suspension but in solution. It is a common experience that such crystalline material is more soluble in a solution of the accompanying "impurities" than in the solvent used; this accounts for the fact that purification by recrystallisation tends to become progressively easier as the fluid material is proportionately reduced. In this business it is the first step that counts (and may lose) most.

For this first step there are also available chromatographic methods. Elegant and effective though these so often are, for a particular problem under consideration they involve a search for the right adsorbent and eluent, as well as several manipulative stages. What the chemist wants in the circumstances is a simple method that is so rapid as to reduce to a minimum waste of time in examining what may ultimately turn out to be crystalline material of no interest. (In work connected with the extraction of "active principles" from vegetable drugs—plants, leaves, roots—it is surprising how often the first crop of crystalline matter was found to be ammonium oxalate!)

It therefore seems that a description might with advantage be given of a procedure first shown to me many years ago by the late Dr. Frank Tutin and now modified in two directions. On various occasions during the past twenty years the procedure has been described or demonstrated to colleagues working in the laboratory and the matter has generally been treated with indifference or scepticism, but recently Dr. C. H. Gray (private communication) has used the technique for recovering crystalline stercobilin hydrochloride from faeces. By this means he has on occasion been able to improve the yield of pure material by as much as 30 per cent.

Although the mechanism of the procedure was never explained by Tutin—a man, in any event, not over-much given to theorising—the experiments described below make it clear that it is merely an application of those phenomena of diffusion so brilliantly exploited by Conway for quantitative micro-analysis. In essence the device involves separating "gum" from crystals on porous plate, using a solvent and yet never wetting the mixture with the solvent.

In the original technique a fragment of unglazed plate or tile—from 1 to 4 or 6 sq.cm. in area, and of any outline, but preferably as flat as possible,—was selected from the pieces of plate produced by dropping one on a hard surface. A quantity of the mixed crystals and gum is spread uniformly on the fragment, in a layer a few mm. thick, and the fragment is laid ("butter-side up") on a smooth surface, such as a glazed tile or a sheet of glass. An ordinary glass funnel is inverted over the fragment, with the stem upwards and open to the air. Solvent from a wash-bottle is then blown round the outside of the funnel where this makes contact with the smooth tile or glass, so as to give a sealing ring of solvent (Fig. 1). When this has evaporated beyond a certain point, the ring will be broken and must be replenished with more solvent. It will be found that after a comparatively short time, depending on the solvent chosen, the solubility of the gum, the viscosity of the mixture, the relative amounts of gum and crystals and so on, the gum has penetrated into the porous plate, leaving the crystals more or less "high and dry" on the surface and ready for further manipulative treatment. Ordinary recrystallisation, for example, can now be applied, especially on the micro scale, with every prospect of preventing precious material from eluding the investigator altogether. Furthermore, after the crystals have been scraped off the porous plate, the gummy material—often the more interesting constituent for further chemical study—can be recovered by simply extracting it from the porous plate in a Soxhlet or other suitable apparatus.

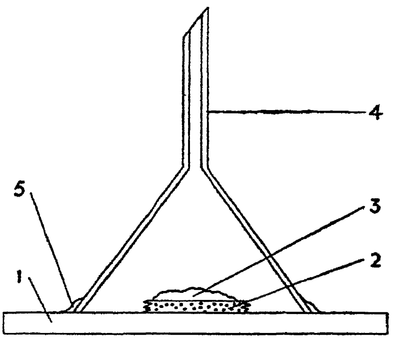

Fig. 1

1. Smooth tile. 2. Porous plate. 3. Mixture under treatment. 4. Glass funnel. 5. Solvent "seal."

The chief objections to this procedure as formerly practised, and possibly as originally devised, by Tutin are two: first, waste of solvent, because of continuous evaporation; secondly, and more important, the need for frequent renewal of the solvent and thus for continuous attention, so that ordinarily the process cannot be allowed to continue without aid overnight. As clearance may be achieved in two or three working days, say, 24 hours, continuous action overnight should save one or two days.

It seemed reasonable to assume that similar results might be achieved on a micro-scale by use of the Conway diffusion unit. The only material available for putting this to practical test was some commercial "carotene paste," consisting of a mass of small carotene crystals embedded in a greenish semi-fluid matrix. The fluid portion could pass through a porous plate without the presence of any solvent, but it took many days to do so, and the residual crystals were by no means cleanly separated. If, however, the tile fragment and the attached carotene paste were put in the inner chamber of a Conway unit, the outer chamber being charged with a little acetone and the whole unit covered with the usual ground glass square plate, almost complete separation was observed in 24 hours or less, without any attention for replenishing the solvent. The Conway unit is, of course, designed to secure maximum diffusion with minimum loss by evaporation, and that is exactly what is wanted for separations of this kind.

For larger scale work the method is still applicable by using an arrangement of two glass cylindrical flat-bottomed evaporating dishes, one inside the other, with an annular space between, to give what is in fact a macro Conway unit that can be taken apart. Using the same carotene paste, I found that ether was much more rapid and effective in "forcing" the gum into the plate than was acetone, although evaporation losses with this set-up may be relatively as well as absolutely rather greater than with the Conway micro unit. Nevertheless, three or four such arrangements of two dishes, with a suitable solvent and pieces of tile of about 10 sq.cm., would probably allow a gram or more of carotene to be separated overnight from the paste used.

The process, like recrystallisation and chromatography, can be carried out again on partly purified material, but it is so effective in cleaning up even the most unpromising mixtures that the investigator who uses it will generally be able to proceed at once to those final stages of purification necessary before any quantitative analyses or physico-chemical tests can be properly undertaken.

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The Identification of Phenol from Synthetic and Natural Sources

By E. J. BOORMAN, C. G. DAUBNEY, AND A. E. MARTIN

A NUMBER of specimens of phenol, derived from three sources, namely (1) alkali fusion of benzenesulphonic acid, (2) high temperature hydrolysis of chlorobenzene, and (3) fractionation of coal tar, have been examined in order to find whether these three types of phenol could be distinguished analytically with certainty, it being assumed that the material may be so discoloured as to prevent the use of the simple coloration test by passing of air.

Each class of sample was always found to contain a small but identifiable quantity of characteristic impurity which was absent from samples of different origin. These impurities were, for class (1), *o*- and *p*-hydroxydiphenyls; for class (2), diphenyl ether; for class (3), naphthalene. The *p*-hydroxydiphenyl was usually isolated and identified by analysis (Found: C, 84·4; H, 5·7. Calc.: C, 84·7; H, 5·9 per cent.) and by the melting-point, 164° C., not depressed by admixture with an authentic specimen; but in general the identification was made by observing the infra-red spectrum of a solution of the residue in carbon tetrachloride.

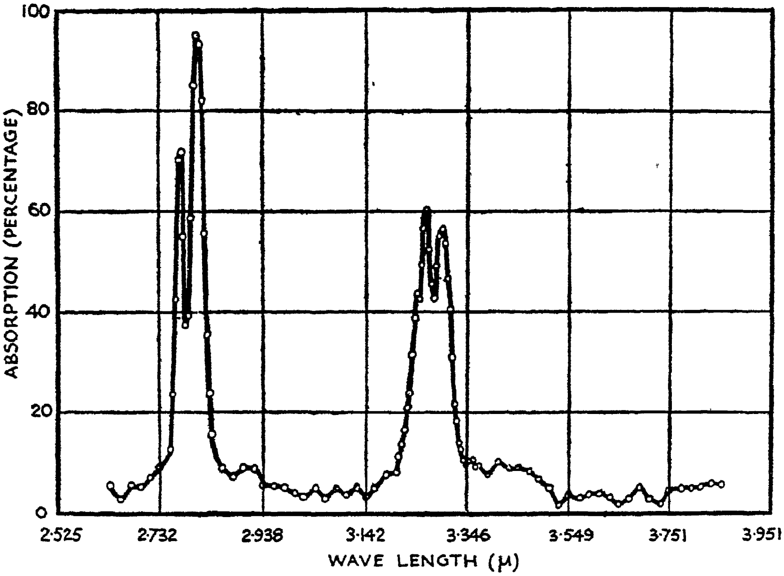

Fig. 1.

PROCEDURE (A)—Distil the sample at 1 to 1·5 mm. pressure (bath at 58° to 60° C.) until distillation ceases. Take up the residue from 1 kilo in ether, filter, evaporate and heat the residue at 100° C. in a current of air to remove traces of phenol. The yield of residue depends on the quality of the original phenol.

Materials of class (1) yield from 0·3 g. to 10 g. of residue, consisting largely of *o*- and *p*-hydroxydiphenyls in the ratio of approximately 4 to 10 parts of *o*- to 1 part of *p*-isomeride. Materials of class (2) or (3) leave not more than a trace of residue. Separate tests showed that added *p*-hydroxydiphenyl in amounts down to 0·02 per cent. can be recovered by this procedure.

PROCEDURE (B)—Dissolve 500 g. of the sample in 800 ml. of sodium hydroxide solution (30 per cent. w/v) and distil in a current of steam until 200 ml. have been collected. Add 80 ml. of the alkali to this, and repeat the steam distillation, collecting 100 ml. and allowing the condenser to become warm towards the end of the operation. Extract the final distillate with 20 ml. of carbon tetrachloride and examine the infra-red spectrum of the solution.

Materials of class (2) show the presence of a trace up to 13 mg. of diphenyl ether from 0·5 kilo of material. Experiments show that added *o*-hydroxydiphenyl does not appear in this distillate. Materials of class (3) yield from an identifiable trace to 10 mg. of impurity from 200 to 500 g. of sample and infra-red examination reveals the presence of naphthalene.

INFRA-RED EXAMINATION—A grating spectrometer was used (compare Fox and Martin[1]), the solution, in an absorption cell 1 cm. or 5 cm. long, being compared with pure carbon tetrachloride in a second similar cell. For identification and estimation of the hydroxydiphenyls the OH band at 2·77 μ was used for the *p*-isomeride, and for the *o*-isomeride a double band having the weaker limb at the same point. The C–H bands of phenol and *p*-hydroxydiphenyl at 3·283 μ. and 3·295 μ. respectively are distinguishable from each other and also from the related bands for *m*- and *o*-hydroxydiphenyls. Bands in the region of 3·3 to 3·5 μ. are not useful, being obscured by absorption due to oily impurities.

Fig. 1 shows a curve for the residue from a phenol of class (1) with the split-off band characteristic of *o*-hydroxydiphenyl (80 per cent. of *ortho*, 20 per cent. of *para*).

Fig. 2 shows the diphenyl ether bands at 3·256, 3·288 and (weaker) 3·298 μ. in residues from phenols of class (2).

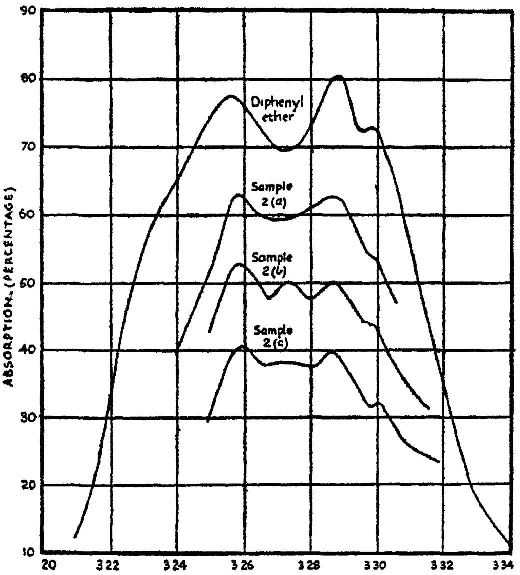

Fig. 2.

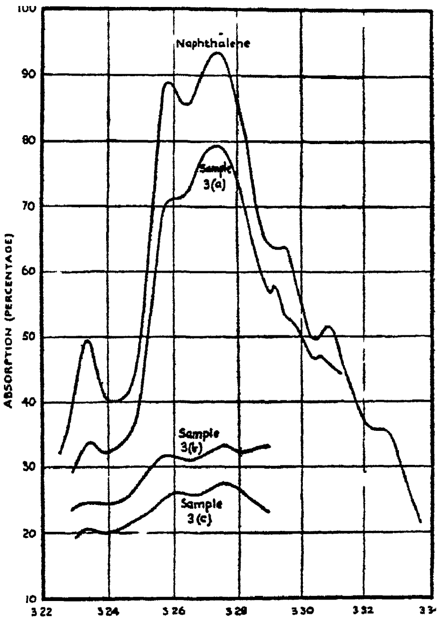

Fig. 3.

Fig. 3 shows the identification of naphthalene in residues from phenols of class (3) the main peaks being at 3·234, 3·259 and 3·276 μ.

The authors are indebted to the Government Chemist for permission to publish this work.

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January, 1947

Reductometric Determination of the Sulphoxide and Amine Oxide Groups

By ERICA GLYNN

(*Read at the Meeting of the Society on February 5th,* 1947)

FROM the analytical point of view the presence of the sulphoxide or amine oxide groups has hitherto been determined indirectly from the figures for the ultimate analysis of the substance under investigation. A direct method is clearly desirable for the sake of accuracy and in order to distinguish, for example, a dioxide from the isomeric sulphide sulphone.

The problem is similar to that of the nitro and azo groups, and similar methods should, therefore, be applicable. For these groups reduction with an excess of stannous chloride[1] or titanous chloride[2] is commonly used and the excess titrated back with standard iodine or ferric alum. Stannous chloride and ferric alum have been adopted in the present work, for iodine is unsuitable in presence of organic sulphides.

Choice of an indicator—The end-point in presence of thiocyanate was found not to be very sharp and an oxidation-reduction indicator was, therefore, adopted. The oxidation-reduction potential concerned lies between 0·55 and 0·22 volt.[3] Nile Blue, Lauth's Violet and Brilliant Cresyl Blue were tried and rejected owing to the dependence of the colour on acid concentration. Potassium indigo trisulphonate[4] was finally chosen (value of $\pi_0 = 0{\cdot}332$), as the passage of the final blue to a deep blue with a reddish tinge, as seen in artificial light, was sharp and easily recognisable. (This characteristic reddish tinge is not observed in absence of artificial light. It is sufficient to place an electric lamp directly behind the flask during the titration.)

METHOD

REAGENTS—Water and acid used for the preparation of all reagents should be boiled free from air and saturated with carbon dioxide.

Standard iodine solution—Approximately 0·1 *N*, standardised against potassium iodate in the usual way.

Standard stannous chloride solution—Approximately 0·2 *N*. Dissolve 54 g. of stannous chloride in 150 ml. of concentrated hydrochloric acid and 1850 ml. of water. Preserve under carbon dioxide and syphon direct into the burette. Standardise by titration against iodine under carbon dioxide with starch as an indicator.

Ferric alum solution—Approximately 0·1 *N*. Dissolve 100 g. of ferric ammonium alum in 400 ml. of 5 *N* sulphuric acid and 1600 ml. water. Filter after it has stood overnight and keep at least a week before use. Standardise this solution by adding an excess of stannous chloride and titrating with iodine. The oxygen equivalent of 1 ml. of 0·1 *N* ferric alum is 0·0008 g.

PROCEDURE—(1) In titrating the stannous chloride with the ferric alum solution take 20 ml. of diluted hydrochloric acid (1+1), with carbon dioxide bubbling, add 10 ml. of stannous chloride from the burette, 5 to 10 drops of 1 per cent. potassium indigo trisulphonate solution and 10 ml. of water and titrate in the cold with ferric alum solution, shaking thoroughly after each addition in the last 0·5 ml. and allowing 5 to 10 seconds for any change of colour, until one further drop causes no further deepening in the final reddish blue. The result agreed with the iodimetric titration within 0·1 per cent.

(2) In titrating a sulphoxide, introduce 20 ml. of an air-free solution in water or alcohol of the substance, containing approximately 0·5 g. of reducible oxygen per litre, into a 250-ml. conical flask on a hot plate, passing carbon dioxide throughout the operation, and add 10 ml. of stannous chloride from the burette. Now add 10 ml. of hot concentrated hydrochloric acid and boil gently for 45 minutes, adding a further 25 ml. of hot water and 5 ml. of concentrated acid after 15 minutes. Finally add 20 ml. of concentrated hydrochloric acid, 10 ml. of water and 5 to 10 drops of 1 per cent. potassium indigo trisulphonate solution and titrate as above with ferric alum. Carry out a blank titration with 20 ml. of air-free water in place of the solution of the substance, heating as before.

Correction of the blank titration—The blank titration is essential, as some oxidation of stannous chloride by atmospheric oxygen is inevitable. It is safe to assume that the reduction of the sulphoxide is largely completed in a very short time and the actual amount of stannous chloride subsequently liable to oxidation is the excess then left in solution. Using the simple blank estimation, the results were in fact found to be low (92 to 98 per cent. of the calculated figure).

Ingold and Smith,[5] in titrating the nitro group with titanous chloride, adopt the device of adding, in their blank experiment, only the amount of reducing solution found to be in excess. The method used here is to make a correction of the blank titration based on the same principle. It has the advantage that the blank determination need not then be repeated for each titration.

If a constant volume, n ml., of stannous chloride solution is always used and titrations are in ml. of standard ferric alum solution, then if titration (cold) without substance is v_c, and titration with heating for a definite period without substance is v_h, whilst titration with heating for the same period with substance is v_s, the apparent titre for the substance (blank uncorrected) is $v_h - v_s$. The true value should be the apparent titre $(v_c - v_s)$ less the blank correction $\times(1 -$ fraction of stannous chloride used up in the reduction of the substance). That is to say, the true titre X is given by

$$X = (v_c - v_s) - \left[(v_c - v_h)\left(1 - \frac{X}{v_c}\right)\right],$$

which gives

$$X = \frac{v_c(v_h - v_s)}{v_h}.$$

As the total hot blank $v_c - v_h$ approaches zero, so the true value of X approaches $v_h - v_s$. The application of this device is shown in Table I with dithian dioxide as the substance analysed.

TABLE I

19·965 ML. OF A SOLUTION OF 2·6964 G. (0·01772 G.-MOL.) OF 1:4-DITHIAN DIOXIDE IN 1 LITRE, TITRATED WITH 0·09977 N FERRIC ALUM

v_c ml.	v_h ml.	v_s ml.	X ml. (calcd. 14·18)	Peroxidic O% found (calcd. 21·02)
25·76	25·21	11·30	14·21	21·06
25·76	24·33	10·92	14·20	21·04
25·76	24·33	11·01	14·10	20·91
25·76	24·33	10·87	14·25	21·13
21·32	19·63	6·53	14·21	21·06

Results with a number of sulphoxides or sulphones are shown in Table II.

TABLE II

Substance	Total O% (Calcd.)	Peroxidic oxygen	
		Found %	Calcd. %
α-Dithian dioxide	21·02	21·04	21·02
Dithian monoxide	11·74	11·77	11·74
Dithian trioxide	28·54	9·41	9·51
Dibenzyl sulphoxide	6·95	6·87	6·95
Dibenzyl sulphone	12·99	0	0
α-Phenylene-1:3-dimethyl disulphoxide	15·82	15·82	15·82
α-2:5-Dimethyl-thiolbenzoic acid dioxide	25·98	12·95	12·99

Amine oxides—The determination was carried out as for sulphoxides. Amine oxides are often most conveniently isolated as picrates and for the analysis of these salts the picric acid must first be removed.

To the solution of an amine oxide picrate in alcohol, add alcoholic potassium hydroxide, warm and allow to stand overnight. Filter into a 100-ml. flask, wash the precipitate with absolute alcohol, make up to 100 ml. with water and take aliquot portions for analysis.

The results for two amine oxides are given below:

Substance	Total oxygen Calcd. %	Peroxidic oxygen	
		Found %	Calcd. %
Dimethylaniline oxide picrate	34·9	4·37	4·28
Diphenylpiperazine α-dioxide octahydrate ..	38·7	7·76	7·73

SUMMARY

A method is described for the determination of sulphoxide and amine oxide groups by quantitative reduction with excess of stannous chloride and back titration with ferric alum, using potassium indigo trisulphonate as an indicator. Results are given for some sulphoxides, sulphoxide sulphones, amine oxide and amine oxide picrate; the average error was less than 1 per cent.

The author's thanks are due to Dr. G. M. Bennett for suggesting this investigation.

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Determination of Sulphate in Sodium Dichromate

BY A. W. WYLIE

FOR the determination of sulphate in the presence of sexivalent chromium compounds, it is usually recommended that chromium be reduced to the tervalent state before precipitation of sulphate with barium chloride. Various combinations of acids and reducing agents have been proposed. Haring and Barrows[1] used hydrochloric acid and ethyl alcohol. Willard and Schneidewind[2] recommended hydrochloric acid and either ethyl or methyl alcohol, hydroxylamine hydrochloride or hydrogen peroxide. Acetic acid and formaldehyde were suggested by Nikitina and Babaeva[3] and by Rabovskii and Raskatova.[4] Macchia[5] advocated the use of acetic acid and hydroxylamine.

The method proposed by Haring and Barrows for the determination of sulphate in chromic acid yields low results unless acetic acid is added before precipitation of barium sulphate. The addition of acetic acid was proposed by Willard and Schneidewind, after Weinland[6] had shown that acetic acid displaces non-precipitable sulphate groups from complex chromium ions and thus brings about almost complete precipitation of sulphate by barium chloride. Most subsequent experimenters have added acetic acid to the solution, whether hydrochloric acid was present or not. In the course of experiments to find whether small amounts of sulphate could be determined in sodium dichromate with the same accuracy as in chromic acid, it has been found (1) that the method of Willard and Schneidewind is equally applicable to sodium dichromate; (2) that increasing proportions of hydrochloric acid increase the quantity of barium sulphate precipitated in the absence of acetic acid; (3) that sulphate may be satisfactorily determined in sodium dichromate without the addition of acetic acid provided 2·7 times the theoretical requirement of hydrochloric acid is used, and the solution is allowed to stand for 15 hours. It has also been found that the presence of large amounts of acetic acid in hydrochloric acid solutions of chromic chloride may cause high results, although it is more usual to obtain results which are slightly low.

To determine approximately 1 per cent. (dry basis) of sodium sulphate in sodium dichromate the procedure of Willard and Schneidewind for the determination of sulphate in chromic acid may be varied as follows:

A 1 to 2 g. sample should be taken and the procedure described below followed; the volume of hydrochloric acid (sp. gr. 1·178) added should be 1·3 times the theoretical requirement and the volume of glacial acetic acid used should be equal to the volume of hydrochloric acid. The precipitation volume should be 500 ml. The time of standing should be 15 hours for the best results (error approx. —0·5 per cent.) although fairly satisfactory results may be obtained by doubling the volume of acetic acid and allowing the solution to stand 1 hour (error approx. —1·5 per cent.). To determine sodium sulphate in sodium dichromate without the addition of acetic acid, a 1 to 2 g. sample should be used and 2·7 times the theoretical requirement of concentrated hydrochloric acid must be added. If the procedure is then carried out as before, the time of standing being 15 hours, the accuracy of the results is equal to that obtained in the presence of acetic acid.

EXPERIMENTAL

AnalaR sodium sulphate and twice recrystallised sodium dichromate were used in all experiments. Solutions of these reagents were standardised, the former by determination of sulphate as barium sulphate and the latter by titration with ferrous ammonium sulphate, using barium diphenylamine sulphonate as indicator. Mixed aliquots of these solutions were acidified with hydrochloric acid (sp.gr. 1·178), or hydrochloric acid and glacial acetic acid, and 50 ml. of ethyl alcohol were added. If the weight of sodium dichromate exceeded 1 g., 70 ml. of alcohol was used. Chromium was completely reduced by heating this solution, of total volume 200 ml., for 30 minutes at 90° to 95° C. The solution was then diluted to 500 ml., heated to 90° C. and treated with 20 ml. of 10 per cent. barium chloride solution added dropwise from a burette. During precipitation, and for periods of standing up to 4½ hours thereafter, the solutions were maintained at 90° C.; for standing periods of 15 hours or more, the solutions were kept at 90° C. for 1 hour only and then cooled to room temperature. The precipitated sulphate was filtered on a No. 42 Whatman paper, thoroughly washed with hot water and ignited at 800°C. Blank tests showed no sulphate in any of the reagents employed The ignited precipitates were white or only slightly discoloured when cold but usually showed a faint yellowish green colour when hot. Each determination was made in duplicate and the mean values are recorded in Tables I to III. When the error in the mean weight of sodium sulphate found did not exceed ±5 per cent. the maximum variation of duplicates from their mean was ±0·2 mg. and the average variation 0·08 mg. When the error in the mean weight of sodium sulphate exceeded ±5 per cent., agreement between duplicates was less satisfactory. An average value, however, is recorded for purposes of comparison.

EFFECT OF HYDROCHLORIC AND ACETIC ACIDS ON THE PRECIPITATION OF BARIUM SULPHATE IN PRESENCE OF CHROMIUM SALTS

TABLE I

In each experiment 5·00 g. of $Na_2Cr_2O_7$ and 0·0546 g. of Na_2SO_4 were present in a final volume of 500 ml.

Expt.	Hydrochloric acid* ml.	Acetic acid ml	Standing period hrs.	Na_2SO_4 found g.	Error mg.	Error per cent.
1	12·5	10	15	0·0540	−0·6	−1·1
2	14·0	10	15	0·0540	−0·6	−1·1
3	15·0	10	15	0·0541	−0·5	−0·92
4	16·0	10	15	0·0544	−0·2	−0·37
5	16·0	10	2	0·0517	−2·9	−5·3
6	13·0	25	15	0·0556	+1·0	+1·8
7	13·0	50	15	0·0553	+0·7	+1·3
8	13·0	75	15	0·0554	+0·8	+1·5
9	12·5	nil	15	0·0247	−29·9	−54·8
10	25·0	nil	15	0·0510	−3·6	−6·6
11	35·0	nil	15	0·0540	−0·6	−1·1

* Volume of hydrochloric acid theoretically required to convert $Na_2Cr_2O_7$ to NaCl and $CrCl_3$, 13·0 ml.

Experiments 1 to 4, Table I, lend no support to the contention of Willard and Schneidewind that decreasing concentrations of hydrochloric acid cause an increase in the quantity of barium sulphate precipitated from solutions of chromium salts in presence of hydrochloric and acetic acids, and suggest that the contrary may be true. Experiment 5 emphasises the necessity of allowing solutions of the concentration used to stand for longer than 2 hours. Experiments 6 to 8 show that high results may be obtained when larger amounts of acetic acid are added. Experiments 9 to 11 indicate that a fairly reliable result may be obtained in absence of acetic acid if the quantity of hydrochloric acid added is 2·7 times the theoretical requirement, excess hydrochloric acid up to this total producing a marked increase in the quantity of barium sulphate precipitated. This optimum quantity of hydrochloric acid may be compared with that recommended by Haring and Barrows for the determination of sulphate in chromic acid, *viz.*, approximately 1·5 times the theoretical requirement.

Experiments 1 and 3 (Table II) reveal no increase in the quantity of barium sulphate precipitated in presence of acetic acid when the concentration of hydrochloric acid is lowered. The smaller concentration of chromium in solution appears to convert a positive error of the order of 1 to 2 per cent. which might have been expected when the volume of acetic acid is twice that of hydrochloric acid (Table I, Experiment 6) to a negative error of the same order (Table II, Experiment 1). Experiments 5, 7 and 9 confirm the results of Experiments 9 to 11,

Table I. It is also shown (Experiments 6 and 8) that prolonged standing greatly increases the quantity of barium sulphate precipitated in absence of acetic acid, particularly at lower concentrations of hydrochloirc acid. It seems reasonable to assume that almost all sulphate would be precipitated under these conditions if sufficient time were allowed. In presence of acetic acid the time factor is of less importance.

TABLE II

In each experiment 2·0 g. of $Na_2Cr_2O_7$ and 0·0219 g. of Na_2SO_4 were present in a final volume of 500 ml.

Expt.	Hydrochloric acid* ml.	Acetic acid ml.	Standing period hrs.	Na_2SO_4 found g.	Error mg.	Error per cent.
1	5·2	10	15	0·0216	−0·3	−1·4
2	5·2	10	1	0·0216	−0·3	−1·4
3	7·0	10	15	0·0218	−0·1	−0·46
4	7·0	10	4·5	0·0217	−0·2	−0·91
5	7	—	15	0·0092	−12·7	−57·9
6	7	—	64	0·0198	−2·1	−9·6
7	8	—	15	0·0152	−7·7	−35·2
8	8	—	40	0·0212	−0·7	−3·2
9	14	—	15	0·0217	−0·2	−0·9
10	14	—	2	0·0199	−2·0	−9·1

* Theoretical requirement of hydrochloric acid 5·2 ml.

TABLE III

In each experiment 1·0 g. of $Na_2Cr_2O_7$ and 0·0219 g. of Na_2SO_4 were present in a final volume of 500 ml.

Expt.	Hydrochloric acid* ml.	Acetic acid ml.	Standing period hrs.	Na_2SO_4 found g.	Error mg.	Error per cent.
1	3	10	4·5	0·0218	−0·1	−0·46
2	3	10	15	0·0221	+0·2	+0·91
3	7	10	4	0·0217	−0·2	−0·91
4	3	—	15	0·0103	−11·6	−53·0
5	5	—	15	0·0164	−5·5	−25·1
6	7	—	15	0·0218	−0·1	−0·46
7	7	—	2	0·0192	−2·7	−12·3

* Theoretical requirement of hydrochloric acid 2·6 ml.

The results in Table III confirm previous findings. The high result in Experiment 2 is attributed to the relatively large volume of acetic acid present. No obvious explanation is apparent for the high results obtained in the presence of larger quantities of acetic acid but such results could be caused by occlusion of greater quantities of chromium or barium salts in the precipitates.

The assistance of Miss J. Mather in carrying out the experimental work involved in this investigation is gratefully acknowledged.

SUMMARY

A method for the determination of small quantities of sodium sulphate (1 per cent.) in sodium dichromate is described. An amount of hydrochloric acid equal to 2·7 times the theoretical requirement is added and the dichromate reduced to a mixture of chromic chloride and sodium chloride by heating with excess of ethyl alcohol. Sodium sulphate in the resulting solution is then precipitated as barium sulphate and the solution allowed to stand for 15 hours before filtration. The results, on the average, are 1 per cent. low.

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DIVISION OF INDUSTRIAL CHEMISTRY
COUNCIL FOR SCIENTIFIC AND INDUSTRIAL RESEARCH
MELBOURNE, AUSTRALIA

November, 1946

Notes

THE CHROMIUM TRIOXIDE TEST FOR COCAINE HYDROCHLORIDE

This identity test for cocaine hydrochloride is given in both the British Pharmacopoeia (1932) and in the United States Pharmacopoeia (20th Ed.). The former requires the use of a 1 per cent. solution of cocaine hydrochloride and a 3 per cent. solution of chromium trioxide; while the latter requires the use of a 2 per cent. solution of cocaine hydrochloride and a 5 per cent. solution of chromium trioxide. In neither case is mention made of the temperature at which the test is to be carried out, nor has any modification of the details of the test appeared in any addendum to the British Pharmacopoeia published since 1932. In the test as given in the British Pharmacopoeia it is stated that a precipitate, which dissolves on shaking, is formed on addition of one or two drops of 3 per cent. solution of chromium trioxide to 1 ml. of 1 per cent. solution of cocaine hydrochloride. Further addition of the chromium trioxide solution is stated to produce a permanent precipitate.

We have found that the test, carried out according to the instructions of the B.P , gives a positive result only if the temperature of the solutions is below 35° C., and then only if considerable care is taken to avoid undue mixing of the drop of chromium trioxide solution with the solution of cocaine hydrochloride. At 35° a definite, but slight, precipitate, which dissolved on shaking, was obtained with the second drop of chromium trioxide solution; but at 36° twelve drops were required to give a precipitate and at higher temperatures still more. At 32° a definite precipitate was obtained with one drop of chromium trioxide solution and a permanent precipitate with ten drops. As the temperature was still further reduced the amount of precipitate obtained with one drop became more substantial. At 25° a permanent precipitate was obtained with the sixth drop.

With a 1 per cent. solution of cocaine hydrochloride and a 5 per cent. solution of chromium trioxide a precipitate, soluble on shaking, was obtained with one drop at all temperatures up to 48°; but at 50° eight drops were required to give a precipitate soluble on shaking, whilst twelve drops gave a permanent precipitate. Using a 2 per cent. solution of cocaine hydrochloride and a 5 per cent. solution of chromium trioxide a precipitate was obtained with one drop at all temperatures up to 56°.

For carrying out these tests the solution of cocaine hydrochloride was contained in a short test-tube (3 × ⅝ inch) and the drop of chromium trioxide solution was allowed to fall on the side of the tube just above the surface of the solution and to run down into the solution.

As laboratory temperatures in the tropics are frequently in the region of 36 to 40° C. or higher, the procedure of the British Pharmacopoeia for carrying out this test is inadequate, as an unsatisfactory positive, or a negative, result is obtained at temperatures above 32°. We consider, therefore, that the details of the test should be modified, either in the direction of conforming to the conditions of concentration of the United States Pharmacopoeia, or by stating that the temperature of the solutions shall not exceed 20° C.

We are indebted to the Director, Sudan Medical Service, for permission to publish this note.

Wellcome Chemical Laboratories
Sudan Medical Service, Khartoum

A. J. Henry
Riad Mansour
February 22nd, 1947

Official Appointments

PUBLIC ANALYST APPOINTMENTS

Notification of the following appointments of Public Analysts has been received from the Ministry of Health since the last record in The Analyst (1947, **72**, 152).

Public Analysts		*Appointments*
Branson, V. C. (Deputy)	..	County of Essex and Borough of Hove.
Chalmers, F. G. D. ..	..	Urban District of Solihull.
Dedicoat, H. (Deputy) ..	..	County Borough of Leeds.
Lovett, T. W. (Deputy)	..	County Borough of Oldham.
Whittle, E. G. ..	..	County Borough of Bristol.

OFFICIAL AGRICULTURAL ANALYST APPOINTMENTS

Notification of the following appointments of Official Agricultural Analysts has been received from the Ministry of Agriculture and Fisheries since the last record in The Analyst (1947, **72**, 153).

Official Agricultural Analysts	*Appointments*
Dedicoat, H. (Deputy)	County Borough of Leeds.
Taylor, W. W.	Administrative County of the Parts of Lindsey, Lincolnshire.

STANDARD METHODS FOR TESTING PETROLEUM AND ITS PRODUCTS*

The eighth edition of the Standard Methods includes new methods for the determination of metals in unused lubricating oils, the examination of the tendency of grease to corrode copper, the determination of the resistance of turbine oils to oxidation, the flash-point of liquid asphaltic bitumen and a modification of the Schwartz method of determining tetraethyl lead in motor fuel.

Fifteen of the previous methods have been amended. The amendments include that of the procedure for determining kinematic viscosity in absolute units, for which the use of a 60 per cent. sucrose solution as a primary standard has been discontinued. Minor amendments have been made to methods for determining knock-rating of aviation fuels and of motor fuels. The bromination method for tetraethyl lead in motor fuel has been withdrawn.

STANDARDISED SUBSTANCES FOR SPECTROGRAPHY, CHEMICAL ANALYSIS AND RESEARCH

We have received from Messrs. Johnson, Matthey & Co., Ltd., their Publication No. 1760, which deals with Standardised Substances for Spectrography, Chemical Analysis and Research. One section of this catalogue contains details of the Spectrographically Standardised Substances formerly supplied by Messrs. Adam Hilger, Ltd.

The number of elements listed is 68 although some are available only in the form of oxides or salts. The materials are believed to be the purest obtainable from various research and industrial laboratories throughout the world, whilst many are specially prepared in the suppliers' own laboratories. Some of the problems associated with the control of purity of these substances have already been described in this Journal (D. M. Smith, Analyst, 1946, **71**, 368). In the catalogue an indication is given of the origin of the material, the form in which it is available and, in some instances, the purity attained.

British Standards Institution

A few copies of the following draft Specifications, issued for comment only, are available to interested members of the Society and may be obtained on application to the Secretary, J. H. Lane, 7-8, Idol Lane, London, E.C.3.

Draft Specification prepared by Sub-Committee C/8/1A—Volumetric Mouldblown and Lampblown Glassware.

CH(C)9938—Draft for Interchangeable Conical Ground Glass Joints (Third Revision of B.S. 572: 1934).

Draft Specifications prepared by Technical Committee OSC/21—Marine Animal and Fish Oils.

CJ(OSC)167—Draft for Vitamin D in Oil for Poultry Feeding Purposes (Types 200 and 400).
CJ(OSC)168—Draft for Vitamins A & D in Oil for Poultry and Other Animal Feeding Purposes.
CH(OSC)107—Draft for Whale Oil (Grades 1, 2, 3 and 4) (Revision of B.S.856).

Draft Specifications prepared by Technical Committee FCC/4—Solvents.

CJ(FCC)507—Draft for Trichlorethylene (Revision of B.S.580).

Draft Specifications prepared by Technical Committee OSC/24—British National Committee on Soaps and Fats of the International Commission for the Study of Fats.

CJ(OSC)516—Draft for Iron in Oils and Fats.
CJ(OSC)517—Draft for Soap in Oils.
CJ(OSC)518—Draft for Peroxide Value of Oils and Fats.
CJ(OSC)519—Draft for Specific Gravity and Apparent Density.
CJ(OSC)520—Draft for Solvent.

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* Eighth Edition. Pp. 576, with 152 diagrams. Published by The Institute of Petroleum, 26, Portland Place, London, W.1. 1947. Price 17s. 6d.

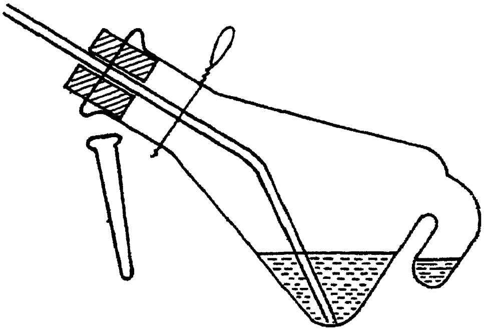
ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Determination of Peroxide Values of Edible Fats and Oils. The Iodimetric Method. C. H. Lea (*J. Soc. Chem. Ind.*, 1946, **65**, 286–291)—It has been shown (Lea, *Ibid.*, 1945, **64**, 106; ANALYST 1945, **70**, 308) that peroxide values determined by the Chapman and McFarlane procedure (*Canadian J. Res.*, 1943, **21**B, 133), in which the fat is heated at 50° C. with ferrous ammonium sulphate and ammonium thiocyanate in 96 per cent. acetone solution and the ferric thiocyanate produced is measured photometrically, are reduced by about 75 per cent. when the reagents are de-aerated before use and the reaction is carried out in an inert atmosphere. Apparently, further oxidation of the fat (or reagent) occurs in presence of free oxygen to a degree roughly proportional to the amount of peroxide already present, probably as the result of a chain reaction initiated by the peroxide. The method used by Bolland *et al.* (*Trans. Inst. Rubber Ind.*, 1941, **17**, 29) depending upon the same principle under different conditions is affected similarly, and attention was therefore directed towards this possible source of error in the iodimetric method, the numerous variations of which have in common the reaction of the fat with potassium iodide in glacial acetic acid and chloroform solution and the subsequent titration of the liberated iodine with sodium thiosulphate solution.

In spite of the replacement of air in the reaction vessel by inert gas in some recent modifications of this method, the iodimetric procedure has been considered somewhat empirical, different procedures giving different results, which vary also with the amount of sample taken. This latter effect has been attributed to re-absorption of part of the liberated iodine by the fat, and attempts to minimise it include progressive reduction of the size of sample taken as the peroxide value increases, so that the titration is kept below 10 ml. of 0·002 *N* sodium thiosulphate (Lampitt and Sylvester, *Biochem. J.*, 1936, **30**, 2237), and estimation of the residual potassium iodide instead of the liberated iodine (Gangl and Rumpel, *Z. Unters. Lebensm.*, 1934, **68**, 533; Dastur and Lea, ANALYST, 1941, **66**, 90). It is now shown that de-aeration of the reagent is necessary before it is brought into contact with the fat if further oxidation during the determination is to be avoided. This can be effected by means of the following apparatus.

Fuse a small piece of Pyrex tube of diameter 13 mm. into the base of a 100-ml. conical flask so as to form a side tube of about 3 ml. capacity as shown in the figure. For the introduction of inert gas fit a bent tube passing loosely through a hole in a rubber stopper from which it can be subsequently withdrawn and replaced by the tightly-fitting tapered glass plug. Fix the flask in a notched cork ring so that it can be supported either vertically or in an inclined position inside a can (slotted to accommodate the neck of the flask), which protects the reaction mixture from daylight during de-aeration. The side tube allows the fat or the potassium iodide (or both) to be kept apart from the solvent during de-aeration and solid fats can be melted without heating the reagent.



Investigation of the conditions of the reaction led to the following recommended procedure.

Cold method—Weigh about 1 g. of the oil or fat (less if the peroxide value is expected to exceed 100) into the side tube and support the flask in an inclined position. Introduce into the flask 20 ml. of a mixture of glacial acetic acid and chloroform (3 : 2 by volume) and 1·2 ml. of freshly prepared, saturated potassium iodide solution, and de-aerate the solvent immediately by passing in oxygen-free nitrogen or carbon dioxide for 15 min. Withdraw the gas delivery tube while the gas is still flowing and close the orifice in the stopper by means of the glass plug. Restore the flask to an upright position thereby flooding the sample with solvent, swirl the mixture immediately, and set the flask aside in a dark place for 1 hr. Add 30 to 50 ml. of water and titrate the mixture with 0·002 to 0·1 *N* sodium thiosulphate with vigorous shaking, adding starch indicator as the end-point is approached. Solid fat should be melted by application of a small flame to the side tube immediately before flooding the tube with solvent.

Hot method—The procedure is as for the cold method as far as the completion of de-aeration. The gas delivery tube is then withdrawn, the glass plug is inserted loosely, and the flask is heated in boiling water until chloroform vapour condenses on the plug, which is then forced home. The flask is immediately placed in a bath at 77° C. for 2 min., and cooled under the tap, and the contents are diluted and titrated as in the cold method.

Since it has hitherto been believed that the most probable sources of error in the iodimetric procedure have been incomplete reaction of the more stable peroxides with the reagent, possible decomposition of very labile peroxides in side reactions, and loss of part of the liberated iodine by re-absorption, the tendency has been to consider the highest values obtained as most nearly correct. The observation that in the iodimetric method, as in the ferrous salt method, further oxidation occurs during the determination indicates that the values given by both methods must frequently have been in error. The ratio between the results of the "aerobic" and "anaerobic" methods is smaller for the iodimetric method (1 to 1·5) than for the photometric ferrous salt method (about 4). This may be due to the difference in the concentration of fat used in the two methods and perhaps to the presence of iron in the latter method. The highest peroxide value obtained is therefore likely to be most nearly correct only when free oxygen is adequately excluded during the determination. Although results obtained by the recommended procedures cannot be regarded as absolutely correct, a number of the more obvious errors of the earlier procedures have been eliminated. A. O. J.

Study of Rancidity of Olive Oils. S. A. Kaloyereas (*J. Amer. Oil Chem. Soc.*, 1947, **24**, 39)—The Wheeler test for peroxides proved relatively the most reliable for assessing rancidity, agreeing more closely with organoleptic tests than did other tests considered. The Issoglio test was of no value for the purpose, but can be of use in differentiating natural olive oils from refined ones. This test (Kerr, R. H., *Ind. Eng. Chem.*, 1918, **10**, 471; Bolton, E. R., *Oils, Fats, and Fatty Foods*, 1928, p. 319) gives values usually below 6 for sweet refined olive oils, and of 12 or over for natural oils.

Rapid Qualitative and Quantitative Determination of Barbiturates from Post-mortem Specimens. P. Valov (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 456)—The prepared material is extracted with aqueous sodium hydroxide solution, the proteins in the extract are coagulated by addition of sodium tungstate and sulphuric acid and are removed by filtration, and the barbiturates are extracted from the filtrate with ether. The yield is complete and the barbiturates are obtained in a crystalline form suitable for qualitative tests and quantitative determination.

Method—Shake 60 ml. of blood or minced viscera with 410 ml. of water and 10 ml. of 10 per cent. sodium hydroxide solution in a litre flask for 5 min. Add 60 ml. of 10 per cent. sodium tungstate solution followed by 60 ml. of 0·67 *N* sulphuric acid, added slowly with continuous shaking, and finally acidify the liquid to Universal Indicator paper with 18 *N* sulphuric acid. Filter the mixture and collect 450 ml. of extract, add an equal volume of ether and shake for 5 min. Separate the ether layer, remove the solvent by distillation, and weigh the residue, deducting the result of a blank determination. Calculate the amount (mg.) of barbiturate in 100 g. of the original material. To determine the blank correction add 250 mg. of phenobarbitone to 60 g. of liver and extract the mixture in the manner described. The average amount of residue found is 253 mg. Extract 60 g. of the liver in the same way. The average amount of residue found is 3 mg. This established average amount may be deducted in the quantitative determination of relatively large amounts of barbiturate; with small amounts, a colorimetric determination of barbiturate in the residue is advisable. The Rotondaro method (*J. Assoc. Off. Agric. Chem.*, 1940, **23**, 777; ANALYST, 1941, **66**, 70) may be used for final purification of the residue, but this is not always necessary.

Extraction with sodium hydroxide separates the barbiturates effectively from fats and oils, the coagulation of proteins yields a clear solution substantially purified, and the relatively large volume of ether used prevents formation of emulsions and promotes rapid and complete extraction. The residue is usually crystalline and in a suitable form for sublimation for melting-point determination. Secanol residues and, sometimes, nembutal residues are non-crystalline, but the latter become crystalline after evaporation of added water. In practice, the following residual amounts of ingested barbiturates are encountered; for secanol, up to 3 mg. per 100 g. of liver; for nembutal, up to 16 mg. and for phenobarbitone, up to 39 mg. It is only very rarely that barbiturate is accompanied by other substances extractable by ether from an acid medium (*e.g.*, salicylic acid, sedormid, or acetophenetidine), but an abnormal weight exceeding 40 mg. of ingested barbiturate per 100 g. of tissue could not be overlooked and would indicate interference of this type. This makes the method practically specific for commonly used barbiturates. A. O. J.

Method of Estimating Penicillin and other Bacteriostatics. J. Fielding (*Brit. Med. J.*, 1947, i, 136–137)—The method depends on the fermentation of glucose in a serum medium, with acid production and subsequent clotting of the serum as an indicator of growth.

Procedure—Prepare a medium of the following composition: sterile horse serum, 1 part; water, 3 parts; glucose, to give a 1 per cent. solution; and Andrade's indicator to give a 1 per cent. solution.

Transfer to sterile containers and sterilise in a steamer for 30 min. Inoculate the medium with 10 cu.mm. per ml. of medium of a 24-hour old broth culture of the Oxford strain of staphylococcus. Put several 25-cu.mm. portions of the seeded medium in a row on the paraffin-waxed surface of a glass slide and mix the first portion with an equal volume of the penicillin-containing sample. Make serial dilutions along the row by transferring 25 cu.mm. the mixture to the next portion, and so on, leaving the last volume unmixed as a control. Take up each of the mixtures and the control into capillary tubes (2 to 3 cm. long and 1 mm. bore), seal the ends in a flame, and incubate the tubes overnight. Where full growth of the organism has occurred the column of fluid in the capillary tube shows opaque clotted serum, coloured uniformly pink by the indicator; where no growth has occurred, the fluid retains its original transparency. The end-point is generally sharp, one capillary-tube dilution containing clear fluid and the next completely clotted pink serum.

The method was successfully used for the estimation of penicillin in blood serum, cerebrospinal fluid, and urine. F. A. R.

Biochemical

Modification of the Fluorimetric Method of Estimating Riboflavine in Biological Materials. E. C. Slater and D. B. Morell (*Biochem. J.*, 1946, **40**, 644–652)—The method is a modification of that of Najjar (*J. Biol. Chem.*, 1941, **141**, 355), but differs from those previously described by including a test for specificity.

Procedure—Carry out the entire operation in weak artificial light and, if solutions have to be kept, store them in darkness. Into three 50-ml., glass-stoppered measuring cylinders, A, B, and C, pipette 1 ml. of acetic acid. (It is preferable to prepare all solutions in duplicate, and then six cylinders are employed.) Into cylinders A and B, put v ml. of the extract, containing not more than 1·2 μg. of riboflavine, followed by 1 ml. of water in cylinder A, and 1 ml. of riboflavine solution (containing 1·2 μg. of riboflavine per ml.) in cylinder B; put $(v+1)$ ml. of water in cylinder C. To each cylinder add 1 ml. of 6 per cent. potassium permanganate solution, mix, and leave for exactly 1 min. Add 1 ml. of 3 per cent. hydrogen peroxide, agitate gently to expel gases, and add 3 g. of anhydrous sodium sulphate followed by 25 ml. of n-butanol - pyridine (92 vols. : 8 vols.). Immerse the cylinders in water at 45° to 60° C. for a few minutes to raise the temperature to at least 40° C., and then remove them from the bath and shake vigorously to dislodge the cake of sodium sulphate at the bottom. Cool to room temperature, again shake vigorously for 2 min., and transfer the contents to a 50-ml. centrifuge tube. Centrifuge at 2,000 r.p.m. for 4 min., and pipette 20 ml. of the clear, upper layer into thin-walled, soda-glass test tubes containing 3 ml. of ethanol and stoppered with corks covered with tinfoil. Mix, and transfer 20 ml. to the cuvette of the fluorimeter and read the fluorescence, keeping the solution in the light-beam for exactly 30 sec. Return the solution in the cuvette to the test tube when readings have been taken on all three solutions, expose the test tubes to sunlight or to artificial light of uniform intensity for a sufficient time to destroy between 80 and 95 per cent. of the riboflavine, and measure the fluorescence again. Solution C should not change by more than 1 scale reading after exposure to sunlight.

To calculate the results, subtract all the readings after the exposure to sunlight from the corresponding readings before exposure, and from the values so obtained for cylinders A and B subtract the value obtained for the blank solution in cylinder C. Then, if A and B represent the corrected values for the reduction in fluorescence on exposure to sunlight of the solutions in cylinders A and B respectively, $B—A$ is equivalent to the reduction in fluorescence of the added riboflavine, *i.e.*, 1·2 μg., and the riboflavine content of the extract is equal to $\frac{A \times 1{\cdot}2}{B-A}$ μg. The accuracy of the method is about 10 per cent. and the limit of sensitivity about 0·01 μg.

F. A. R.

Fluorimetric Estimation of Riboflavine in Urine. D. B. Morell and E. C. Slater (*Biochem. J.*, 1946, **40**, 652–657)—Seven different methods of estimating riboflavine in rat and human urine were compared. Methods using a hyposulphite ($Na_2S_2O_4$) blank, even without adsorption on Florisil, gave high results because they included "apparent riboflavine." Urine contains not only "apparent riboflavine," but also precursors that are changed into "apparent riboflavine" by sodium hyposulphite - stannous chloride reduction, by potassium permanganate oxidation, or during the process of adsorption on Florisil. The methods of Najjar (*J. Biol. Chem.*, 1941, **141**, 355) were not specific, giving high results for urines of low riboflavine content, and low values for urines of high riboflavine content. No completely satisfactory method for overcoming these difficulties was discovered. F. A. R.

Determination of Carbon Monoxide in Blood and of Total and Active Haemoglobin by Carbon Monoxide Capacity. D. D. van Slyke, A. Hiller, J. R. Weisiger, and W. O. Cruz (*J. Biol. Chem.*, 1946, **166**, 121–148)—The procedure for estimating total haemoglobin comprises: (1) reducing the ferrihaemoglobin to ferrohaemoglobin with sodium hyposulphite ($Na_2S_2O_4$); (2) saturating the haemoglobin with carbon monoxide; (3) extracting the uncombined carbon monoxide and the nitrogen from the solution; (4) adding ferricyanide to liberate carbon monoxide from carboxyhaemoglobin; and (5) extracting and measuring the carbon monoxide thus liberated. For active haemoglobin the procedure is the same as for total haemoglobin except that step (1) is omitted, whilst for estimating the carbon monoxide content of blood the procedure is the same except that step (2) is omitted, and pressure readings are taken at 0·5-ml. volume even when the sample is 2 ml. The following procedure is that used for total haemoglobin with 1-ml. samples, and indication is given of the steps that are omitted when active haemoglobin or carbon monoxide contents are determined. For samples of 2·0, 0·5, 0·2, or 0·1 ml. the procedure is the same as for the 1-ml. sample except that different amounts of reagent are used, as indicated in Table I, and that, when 2-ml. samples are used for total or active haemoglobin the pressure readings are made with the carbon monoxide at 2·0 ml. rather than 0·5 ml. volume.

TABLE I

Vol. of blood sample taken, ml. ..	2	1	0·5	0·1–0·2
Capryl alcohol used, drops . ..	3	2	1	1
Vol. of saponin - borax solution used, ml. ..	4	3	1·5	1·0
$Na_2S_2O_4$ used, mg.*	35 ± 5	35 ± 5	18 ± 2	7 ± 1
Vol. of CO gas in chamber to saturate Hb, ml.† ..	10	10	10	10
Vol. of ferricyanide - acetate solution used, ml. ..	1·5	1·5	1·0	0·5
Vol. of *N* NaOH:				
Measured into cup, ml. ..	3·0	3·0	2·0	2·0
Admitted into chamber, ml.	1·5	1·5	1·0	0·5
Vol. of gas when p_1 and p_0 are read, ml.	2·0	0·5	0·5	0·5

* To determine *active* Hb, omit the $Na_2S_2O_4$. In all other details, the procedure is the same as for total Hb.

† To determine CO *content* of blood as drawn, omit the saturation with 10 ml. of CO. Make pressure readings with the gas at 0·5 ml. volume, even when the sample is 2 ml. Other details, including addition of $Na_2S_2O_4$, are the same as for total Hb.

Method: Step 1—Put 2 drops of capryl alcohol into the cup of the chamber of the Van Slyke - Neill manometric apparatus and draw down most of it into the chamber, leaving enough above the stop-cock to fill the capillary between it and the cup. Pipette 3 ml. of saponin - borax solution (dissolve 1 g. of saponin and 3 g. of borax in 100 ml. of water and add 0·1 ml. of capryl alcohol as a preservative) and draw about 0·5 ml. into the chamber. Draw the blood sample into a 1-ml. pipette calibrated for delivery and fitted at the tip with a rubber ring. Put the tip of the pipette containing the sample through the solution in the cup so that the rubber ring presses on the bottom of the cup and then deliver the blood at a slow, even rate into the chamber, taking about 2 min., until a small bubble of air follows the blood into the capillary beneath. Close the stop-cock and dislodge the bubble at the top of the capillary by means of a fine wire dipped in capryl alcohol. Run a little of the saponin - borax solution into the chamber to wash the blood out of the capillary. Introduce from a calibrated glass spoon 35 ± 5 mg. of powdered sodium hyposulphite into the cup, dissolve in the saponin - borax solution by stirring for a few seconds, and immediately draw the solution into the chamber. Put about 0·5 ml. of mercury into the cup to provide a seal for the tube from which the carbon monoxide gas is next delivered.

Step 2 (omitted in estimating the carbon monoxide content of blood)—Transfer 10 ml. of carbon monoxide from a Hempel pipette into the chamber, lower the mercury meniscus to the 50-ml. mark and alternately admit carbon monoxide at the top of the chamber and mercury at the bottom until the pressure indicated by the manometer has risen to 150 mm. Run the 0·5 ml. of mercury from the cup into the chamber, leaving sufficient to fill the capillary above the stop-cock, lower the mercury in the chamber, to the 50-ml. mark, and shake the chamber for 1·5 to 2 min., but not longer. Avoid exposure of the chamber to direct sunlight during shaking. Eject the excess of carbon monoxide from the chamber. This treatment results in laking of the blood cells by the saponin, reduction of the oxygen originally present in the blood by the hyposulphite, reduction of ferrihaemoglobin to ferrohaemoglobin, and combination of the latter with carbon monoxide to form carboxyhaemoglobin. In addition, the greater part of the nitrogen is extracted from the solution.

Step 3—Seal the stop-cock with mercury, lower the mercury into the chamber to the 50-ml. mark, and shake the evacuated chamber for 2 min. at a rate of 300 to 400 oscillations per minute. Protect the solution from light, eject the extracted gas, and again seal the stop-cock with mercury. The only gases now left in solution are carbon monoxide, in the form of carboxyhaemoglobin, and carbon dioxide.

Step 4—Lower the mercury in the chamber sufficiently to bring the surface of the aqueous solution down into the broad portion of the chamber, and into the cup above the chamber put 0·5 ml. of mercury and 1 or 2 ml. of water. Now introduce into the chamber 1·5 ml. of ferricyanide - acetate reagent (freshly prepared each day by mixing 5 ml. of an acetate buffer solution containing 75 g. of sodium acetate trihydrate in 100 ml. of water, to which are added 15 ml. of glacial acetic acid, with 15 ml. of 32 per cent. potassium ferri-cyanide solution) by means of the pipette with the rubber ring attachment previously described. Seal the stop-cock with mercury from the cup and rinse the cup with water to remove any ferricyanide left in it. Lower the mercury in the chamber to the 50-ml. mark and shake the chamber for 3 mins. to extract the carbon monoxide plus the carbon dioxide liberated from the blood and reagents by the acid in the ferricyanide solution.

Step 5—Admit mercury from the levelling bulb to the chamber until the gas space is decreased to 3·5 to 4 ml. Put 3 ml. of air-free N sodium hydroxide into the cup and at once run 1·5 ml. into the chamber. Admit a few droplets of mercury and then after about 1 min. raise the meniscus to the 0·5-ml. mark and record the reading, p_1, on the manometer and the temperature of the water-jacket. Eject the gas from the chamber, lower the meniscus of the solution below the 0·5-ml. mark, and then raise to the mark and record the reading, p_0.

Blank Analysis—Carry out a blank analysis in precisely the same way, substituting water for the blood. The value of p_1-p_0 thus found is the correction c. With 1-ml. samples of blood this value should not exceed 1·5 mm. in estimating the total haemoglobin or 3·5 mm. in estimating active haemoglobin, provided the reagents have been kept free from air.

Washing the chamber after analysis—In washing the chamber after analyses, it is important to remove all particles of methaemoglobin precipitate from the walls of the chamber. After recording the reading, p_0, and without releasing the vacuum, admit about 10 ml. of water into the chamber, mix with the blood solution, and eject the mixture from the chamber. Fill the cup with N sodium hydroxide, dissolve in this about 70 mg. of sodium hyposulphite, and admit the solution to the chamber followed by about 20 ml. of water. Lower the mercury below the 50-ml. mark and shake with the motor until all particles adhering to the wall are dissolved. Eject the solution from the chamber, wash once with water, and then admit 20 ml. of water and 2 ml. of $2\,N$ sulphuric acid. Shake with the mercury surface in the broad part of the chamber somewhat above the 50-ml. mark. Eject the acid solution and wash twice with 20-ml. portions of water.

Calculation—If P_{CO} be the pressure exerted by the carbon monoxide, then $P_{CO}=p_1-p_0-c$. The result in volumes per cent., or mg.-mols. per litre, or grams of haemoglobin per 100 ml. of blood, is calculated by multiplying P_{CO} by the proper factors given in Tables II or III [in which S = final liquid volume and a = final gas volume]. The amount of inactive haemoglobin is obtained by subtracting the active haemoglobin (without sodium hyposulphite) from the total haemoglobin (with sodium hyposulphite).

TABLE II

FACTORS BY WHICH MM. OF P_{CO} ARE MULTIPLIED TO GIVE BLOOD CO CONTENT

	Volumes per cent. of CO in blood					Mg.-mols. of CO per litre of blood				
Temperature °C.	Sample =2 ml. S=7·5 ml. a=2·0 ml. i=1·00	Sample =2 ml. S=7·5 ml. a=0·5 ml. i=1·00	Sample =1 ml. S=5·5 ml. a=0·5 ml. i=1·00	Sample =0·5 ml. S=3 ml. a=0·5 ml. i=1·00	Sample =0·2 ml. S=1·7 ml. a=0·5 ml. i=1·00	Sample =2 ml. S=7·5 ml. a=2·0 ml. i=1·00	Sample =2 ml. S=7·5 ml. a=0·5 ml. i=1·00	Sample =1 ml. S=5·5 ml. a=0·5 ml. i=1·00	Sample =0·5 ml. S=3 ml. a=0·5 ml. i=1·00	Sample =0·2 ml. S=1·7 ml. a=0·5 ml. i=1·00
15	0·1248	0·03120	0·06240	0·1246	0·3113	0·05569	0·01392	0·02784	0·05558	0·1389
16	43	08	16	41	02	50	87	74	38	84
17	39	0·03097	0·06194	37	0·3091	30	82	64	19	79
18	35	86	72	32	80	10	77	54	0·05499	74
19	30	75	50	28	69	0·05490	72	44	80	69
20	26	63	26	24	57	71	67	34	60	64
21	22	53	06	19	47	51	62	24	40	59
22	18	42	0·06084	15	36	32	57	14	20	54
23	13	31	62	10	25	12	53	05	00	50
24	09	26	41	06	14	0·05392	48	0·02695	0·05381	45
25	04	10	19	02	04	73	43	85	62	40
26	01	00	0·05999	0·1198	0·2993	54	38	76	42	35
27	0·1196	0·02989	78	94	83	36	34	67	24	31
28	92	79	57	89	73	18	29	58	05	26
29	88	68	36	85	62	0·05299	25	49	0·05287	22
30	83	58	15	81	51	79	20	39	68	17
31	79	48	0·05895	77	41	62	15	20	50	13
32	75	37	74	73	31	44	11	21	33	08
33	71	27	54	69	21	26	06	12	15	04
34	67	17	33	65	11	08	02	03	0·05198	0·1299

For samples of 0·1 ml., use 10 times the factors for samples of 1·0 ml.

TABLE III

FACTORS BY WHICH MM. OF P_{CO} ARE MULTIPLIED TO GIVE G. OF HAEMOGLOBIN PER 100 ML. OF BLOOD

Temperature °C.	Sample = 2 ml. S = 7·5 ml. a = 2·0 ml. i = 1·00	Sample = 2 ml. S = 7·5 ml. a = 0·5 ml. i = 1·00	Sample = 1 ml. S = 5·5 ml. a = 0·5 ml. i = 1·00	Sample = 0·5 ml. S = 3 ml. a = 0·5 ml. i = 1·00	Sample = 0·2 ml. S = 1·7 ml. a = 0·5 ml. i = 1·00
15	0·09147	0·02288	0·04575	0·09129	0·2281
16	14	79	58	0·09097	73
17	0·09082	70	40	64	65
18	50	62	23	32	57
19	18	53	06	00	49
20	0·08986	45	0·04489	0·08968	40
21	54	37	73	36	33
22	20	29	57	02	25
23	0·08888	21	42	0·08870	17
24	56	13	26	38	09
25	24	06	11	06	01
26	0·08792	0·02198	0·04396	0·08776	0·2193
27	62	90	80	46	85
28	32	83	65	15	78
29	02	75	50	0·08684	70
30	0·08671	68	35	54	63
31	42	60	19	24	55
32	14	52	04	0·08594	48
33	0·08584	45	0·04290	66	40
34	54	38	75	37	33

For samples of 0·1 ml., use 10 times the factor for samples of 1·0 ml.

F. A. R.

Spectrophotometric Determination of Tyrosine and Tryptophan in Proteins. T. W. Goodwin and R. A. Morton (*Biochem. J.*, 1946, **40**, 628–632)—The ultra-violet absorption spectra of tyrosine and tryptophan in 0·1 N sodium hydroxide intersect at 257·15 and 294·4 mμ (ϵ 2748 and 2375, respectively). By measuring the intensity of absorption at 294·4 and 280 mμ by means of a photo-electric spectrophotometer, mixtures of tyrosine and tryptophan can be analysed with considerable accuracy provided the molar ratios are not greater than 20:1 either way.

The observed intensity of absorption for a 1-cm. cell is given by the expression $yA + (x - y) B$, where x equals the total g.-mol. per litre in solution, y equals the g.-mol. of tyrosine per litre, and A and B are the values of ϵ for tyrosine and tryptophan, respectively, at any wavelength other than a point of intersection. In examining the spectra of protein solutions, irrelevant absorptions at 294·4 and 280 mμ have to be allowed for. The simplest procedure is to extrapolate linearly from 370 and 340 mμ to those wavelengths. Analyses, by this method, of mixtures of tyrosine and tryptophan of known composition, gave recoveries of the two amino acids ranging from 91 to 106 per cent. when the 20:1 ratio was not exceeded. F. A. R.

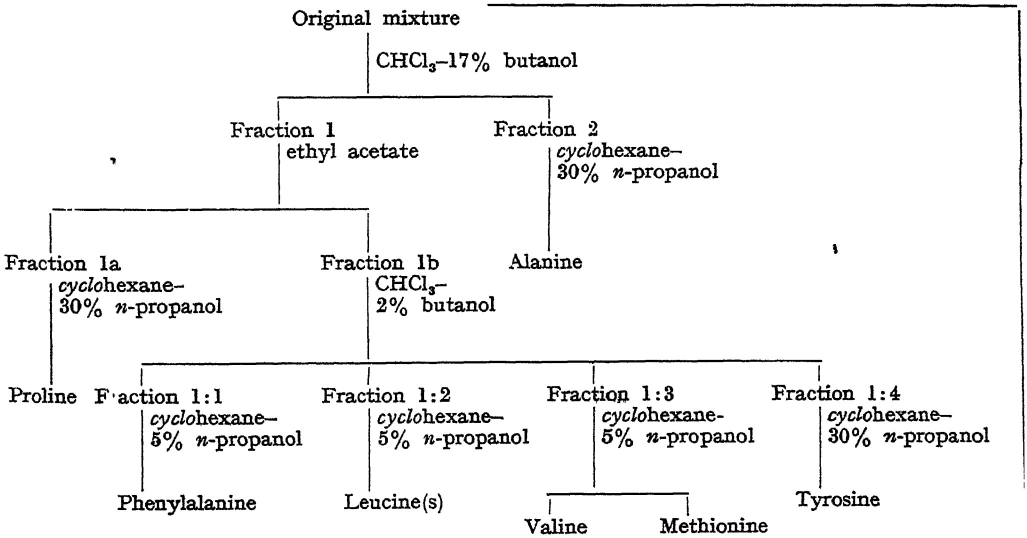
Colorimetric Determination of Pyridoxine, Pyridoxal, and Pyridoxamine. A. A. Ormsby, A. Fisher, and F. Schlenk (*Arch. Biochem.*, 1947, **12**, 79–81)—Pyridoxal, pyridoxamine, and pyridoxine give a bright yellow colour, an orange to pink colour, and an orange colour, respectively, with a solution of diazotised sulphanilic acid. Maximum intensity is reached one minute after addition of the reagent, but the stability of the colours obtained is very limited. The method can be used to estimate the amount of any one of the three substances in a biological preparation, provided the other two substances are absent. The reagent is prepared according to the method of Bina, Thomas, and Brown (*J. Biol. Chem.*, 1943, **148**, 111; ANALYST, 1943, **68**, 256). F. A. R.

Application of Partition Chromatography to the Estimation of the Monoamino Acids in Proteins. G. R. Tristram (*Biochem. J.*, 1946, **40**, 721–733)—Difficulties were encountered in the application of the original method of Gordon, Martin and Synge (*Ibid.*, 1943, **37**, 79), in which separation of acetamino acids was obtained by eluting with various solvents from a column of silica gel in presence of coloured indicators, and a modified procedure was therefore developed; this proved to be satisfactory for the amino acid systems studied. It was shown that the adsorption of acetamino acids on iron-free silica gel is dependent on the water content of the silica.

Procedure—Hydrolyse the protein by dissolution in 5 volumes of concentrated hydrochloric acid at 37° C., heating under reflux in boiling water until frothing ceases, then diluting with water so that the acid concentration is 5·5 *N*, and finally heating under reflux for 24 hours. Remove the excess acid by repeated evaporation under reduced pressure from a water-bath at 45° C.

Adjust the sample to a volume of 2 to 3 ml., acetylate the amino acids by the method of Gordon *et al.* (*loc. cit.*), and extract the acetamino acids from the acetylated mixture by the chromatographic procedure of Gordon *et al.* (*Ibid.*, 1943, **37**, 313), using Alphamine Red-R as indicator. It was found that small amounts of sulphuric acid sufficient to cause esterification and decomposition were being extracted by the eluting solvent. This was overcome by adding 5 ml. of water to the receiver at the beginning of the elution and, when elution was complete, testing a 1-ml. sample for sulphate. If sulphate was present, the aqueous layer was removed in a separating funnel and washed several times with the eluting solvent, all the organic solvent being filtered through a No. 2 Whatman filter paper. It was also found essential to acidify the acetylated mixture to *p*H 1·0 to ensure complete extraction of the acetamino acids. The complete removal of ethanol, butanol, and acetic acid is also extremely important, and is ensured by removing the ethanol under reduced pressure and drying the residue overnight in a vacuum desiccator at 0·005 mm. over anhydrous calcium chloride and potassium hydroxide pellets.

The scheme for fractionation recommended by Gordon *et al.* was found to give low recoveries of proline, so the following procedure was adopted:



F.A.R.

Organic

Quantitative Analysis of Hydrocarbon Mixtures by means of Raman Spectra. E. J. Rosenbaum, C. C. Martin, and J. L. Lauer (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 731–733)—A method is described for the analysis of mixtures containing up to four or five components, without the use of an internal standard, giving an average deviation of less than 2 per cent. The assumption is made that for constant illumination of the sample (usually 15 ml.), the intensity of light scattered by a hydrocarbon in a mixture is directly proportional to the concentration of that hydrocarbon. Special mercury arc lamps giving little continuous radiation were used to reduce background scattering.

Calibration mixtures, each containing the same series of pure components in different proportions, were prepared, and the corrected optical density of each suitable Raman line of each component in a mixture was measured. From the optical density, the effective intensity of each line was calculated, and, using an empirical averaging process, relations between the effective intensity and the concentration were obtained which enabled the analysis of a mixture of these components of unknown composition to be carried out.

The method is illustrated by results obtained in analysing synthetic mixtures of *o*-, *m*-, and *p*-xylene, and ethylbenzene; of *o*-, *m*-, and *p*-ethyltoluene, mesitylene, and pseudocumene; and of the trimethylpentanes. D. A. P.

Determination and Isolation of the Organic Acids in Fruit. F. A. Isherwood (*Biochem. J.*, 1946, **40**, 688–695)—A method was developed for the quantitative separation of many of the acids commonly found in fruit. This can readily be modified to allow the constituent acids to be isolated in a relatively pure state and identified. The acids are extracted from an acidified extract of fruit by means of a 1+1 mixture of *n*-butanol and chloroform, and a concentrated solution of the acids is then prepared in a 1+1 mixture of *tertiary* amyl alcohol and chloroform. The mixture of acids thus obtained is separated into its components by means of a modified partition chromatogram.

Procedure. Extraction of acids—Grind the frozen fruit at −20° C. to a flour and mix 50 g. with 20 ml. of 0·2 *N* sulphuric acid. Allow the mixture to thaw and, if necessary, adjust the *p*H to 2·0 ± 0·2. Centrifuge and decant the supernatant liquid. Add 10 g. of the resulting extract, which should contain organic acids equivalent to 10 to 20 ml. of 0·1 *N* sodium hydroxide, to 12 g. of specially prepared silica gel (see below) and incorporate the liquid into the gel so that a superficially dry powder is obtained. Add about 50 ml. of a 1+1 mixture of *n*-butanol and chloroform, and transfer the mixture to a glass tube, 20 cm. long and 5·2 cm. in diameter. Collect the filtrate, allow the column to run dry, and then fill up the tube with fresh solvent. Collect 350 to 400 ml. of the filtrate, and then a further 50 ml. in a separate receiver; titrate this with 0·1 *N* sodium hydroxide to *p*H 9·4. Not more than 0·2 ml. should be required if extraction has been complete. Titrate the main bulk of the filtrate with 0·1 *N* sodium hydroxide to *p*H 9·4 and leave for half an hour. Separate the lower solvent layer and wash twice with 10 to 15 ml. of water. Concentrate the combined aqueous layers under reduced pressure to about 2 ml. in a water-bath at 35° C., transfer quantitatively to a small beaker, and dry *in vacuo* over phosphorus pentoxide. Add sufficient 2 *N* sulphuric acid to liberate the organic acids, and mix the liquid, which should have a volume of 0·7 to 1·0 ml., with 1·0 g. of silica gel. Add about 10 ml. of a 1+1 mixture of *tertiary* amyl alcohol and chloroform, transfer the mixture quantitatively to a glass tube, 25 cm. long and 1·3 cm. in diameter, and collect the filtrate. Thereafter the procedure is the same as that described above for extraction with the butanol - chloroform mixture. Finally, rinse the beaker with 5 ml. of solvent and transfer this to the tube when the top of the column is dry. Add fresh solvent to the column until 30 ml. of filtrate have been collected. Check the completeness of the extraction by collecting a further 5 ml. and titrating with 0·1 *N* sodium hydroxide to *p*H 8·4; less than 0·1 ml. should be required.

Separation and analysis of a mixture of acids—To 3·0 ml. of 0·5 *N* sulphuric acid add 3·0 g. of silica gel and mix thoroughly. Add 20 ml. of a 35:65 mixture of *n*-butanol and chloroform, pour the suspension into a glass tube (1·3 cm. intern. diam.) and allow the column to pack down. Add to the filtrate at the rate of 0·1 ml. per min. a 0·0004 *M* solution of thymol blue [0·186 g./litre]. Put 2 ml. of a solution of the acids in a 1 + 1 mixture of *tertiary* amyl alcohol and chloroform on to the top of the column and develop the column with a 35 per cent. *n*-butanol - chloroform mixture at the rate of 1 ml. per min., carefully observing the colour of the filtrate. The volume of indicator should be 1/8th to 1/10th the volume of the solvent. The mixture of solvent and indicator is blue when no organic acid is present, changing to green and then to yellow, and finally to pink when each organic acid begins to wash out of the column. Change the receiver at the point when the colour is still greenish-blue and again when the colour reverts to blue. Record the volume of solvent and indicator in the receiver containing each fraction and titrate to *p*H 8·4 with 0·01 *N* barium hydroxide, following the addition of 20 ml. of carbon-dioxide-free water. With a mixture of acids, of which the composition is unknown, a 35 per cent. (v/v) mixture of *n*-butanol and chloroform should be used as the developing solvent, but if, as sometimes happens, an acid fraction comes through the column before the oxalic and malic acid fractions, the chromatographic separation should be repeated and the column developed first with 5 per cent. *n*-butanol - chloroform, if acetic and formic acids have to be separated, and then, after about 100 ml. of solvent have passed through the column, with 10 per cent., with 20 per cent., and eventually with 35 per cent. *n*-butanol - chloroform.

Preparation of silica gel—The method used by Gordon *et al.* (*Ibid.*, 1943, **37**, 79) was found to be unsuitable, and the following method is recommended. Dilute water glass (6 lb.) with twice its volume of warm water, filter twice through hardened filter paper to remove suspended matter, and then add a little methyl orange followed by 10 *N* hydrochloric acid with vigorous stirring. When the mixture is acid, add a further 200 to 300 ml. of hydrochloric acid, leave for several hours, and filter through a hardened filter paper. Suspend the silica in 5 litres of 10 *N* hydrochloric acid, leave overnight, filter, and wash with 10 litres of 5 *N* hydrochloric acid to remove all but traces of aluminium and iron. Wash with about 20 litres of water or until free from chloride, then with 10 litres of absolute alcohol, and finally with 5 litres of dry ether. Leave the dry silica gel for 2 weeks and then suspend it in 7 litres of 10 *N* hydrochloric acid. Leave overnight, filter, and wash with 10 litres of 5 *N* hydrochloric acid, and then with water, ethanol, and ether as before. Store in a closed vessel.

The acids commonly occurring in fruit are washed out of the column in the following order: acetic, fumaric, glutaric, formic, succinic, *trans*-aconitic, malonic, oxalic, tricarballylic, glycollic, malic, citric, and tartaric. The acids were washed off the column most readily by means of the 35 per cent. solvent mixture and least readily by the 5 per cent. mixture. The recovery of individual acids from a mixture of six acids was almost quantitative.

F. A. R.

Identification of Fructose. C. Neuberg and I. Mandl (*Arch. Biochem.*, 1946, **11**, 451–456)—Fructose can be identified in quantities less than 0·00225 g., and can be differentiated from glucose, by the formation of the methylphenylosazone. The reaction can be carried out in presence of glucose, saccharose, and sorbose, and can be used as a micro-reaction. The derivative forms at temperatures as low as 4° C, separates as characteristic crystals (photomicrographs are given), and melts (decomp.) at 158° to 160° C.

Method. Preparation of the methylphenylosazone—To a solution of 4·1 g. of sodium acetate trihydrate in 10 ml. of warm water were added 5·13 g. of methylphenylhydrazine sulphate (from Eastman Kodak), followed by 2 ml. of glacial acetic acid. After cooling to 20 to 25° C., a solution of 1·8 g. of fructose in 2 ml. of water was added. The liquid solidified to a crystalline mass after standing for 1 hour at ordinary temperature. Crystallisation sets in 25 min. after inoculation. Recrystallisation from ethyl acetate, or aqueous methyl Cellosolve, or dioxan, yields long needles. The reaction mixtures are well buffered and have a *p*H of 4·55 to 4·58, at which *p*H saccharose is stable and does not react with the methylphenylhydrazine. In presence of sorbose, resinous masses sometimes separate; this can be remedied by addition of small quantities of alcohol.

For the micro-test, place a small drop of the reaction mixture, by means of a capillary, on a microscope slide with a single concavity, and scratch with a glass thread, evaporation being prevented by means of a cover-glass. In presence of fructose, yellow needles form at room temperature in 40 min. or even less. The yields in the macro-technique are 64–80 per cent. of the theoretical.

The methylphenylhydrazine sulphate can usually be used directly; if the aqueous solution is yellow, recrystallisation from ethyl alcohol and ether may be necessary, but stirring with a mixture of 4 parts of ether and 1 part of absolute alcohol is often sufficient to extract the yellow impurity.

E. M. P.

Micro-Kjeldahl Determination of Nitrogen in Gramicidin and Tryptophan. Comparison of the Gunning-Arnold-Dyer and Friedrich Methods. L. M. White and G. E. Secor (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 457–458)—Hotchkiss and Dubos (*J. Biol. Chem.*, 1941, **141**, 155) reported that the Dumas combustion method is unreliable when applied to the determination of nitrogen in gramicidin, and that consistent results are obtainable with the Kjeldahl method only when the sample is treated with hydriodic acid by the Friedrich method (Pregl and Roth, "Quantitative Organic Micro-analysis," translated by E. B. Daw, 3rd Ed., p. 89, Philadelphia, Blakiston Co., 1937) before digestion. Similar difficulties are encountered in the determination of the nitrogen in tryptophan. Tryptophan constitutes up to 40 per cent. of the gramicidin molecule, and the failure to obtain quantitative yields of nitrogen from these substances may be due to the presence of half the nitrogen of tryptophan in the indole ring. Experience with the Gunning-Arnold-Dyer method as described by Clark (*J. Assoc. Off. Agric. Chem.*, 1941, **24**, 641) suggested that the nitrogen in tryptophan could be completely recovered by this method without the tedious pre-treatment with hydriodic acid.

Procedure—Digest a 5- to 7-mg. sample (weighed by difference) in a 30-ml. Kjeldahl flask containing a glass bead with 1·5 ml. of conc. sulphuric acid, 500 mg. of potassium sulphate, and 40 mg. of mercuric oxide until the liquid becomes clear, and for a further 30 min. Cool, add 1 drop of ethyl alcohol, and heat again until the liquid is colourless. The digests must be boiled vigorously during the later stages of the digestion, otherwise low results may be obtained. Distil the liquid according to the method of Clark (*loc. cit.*), but receive the ammonia in 5 ml. of 2 per cent. by weight boric acid solution containing 1 per cent. by volume of the mixed indicator of Ma and Zuazaga (*Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 280). Titrate the absorbed ammonia with 0·01 *N* hydrochloric acid in a final volume of 25±2 ml. The acid should be standardised by determination of the nitrogen in acetanilide (Nat. Bur. Standards, standard sample 141) or cystine (standard sample 143). Make blank determinations daily.

The authors' modification of the Friedrich method differs from that described by Clark (*loc. cit.*) in the same particulars as does their modification of the Gunning-Arnold-Dyer method, and, in addition, they use red phosphorus with the hydriodic acid as prescribed by Pregl and Roth (*op. cit.*).

The percentages of nitrogen found by the Gunning-Arnold-Dyer method in a number of samples of tryptophan and gramicidin and their derivatives showed no significant difference from those obtained by the Friedrich method. Nitrogen in the carboline ring might be expected to be resistant to oxidation, but analysis of 3: 4: 5: 6-tetrahydro-4-carboline-5-carboxylic acid gave a nitrogen recovery of 99·5 per cent. by the method described. The complete recovery of nitrogen from the indole ring of tryptophan is in accord with the findings of Clark (*loc. cit.*) and other investigators, who demonstrated the applicability of mercury as a catalyst to similar refractory, nitrogen-containing rings, *e.g.*, pyridine, quinoline, purine, pyrimidine, thiazole, and pyrrole. The authors, working with solid samples, have been unable to confirm the results of Miller and Houghton (*J. Biol. Chem.*, 1945, **159**, 373; ANALYST, 1945, **70**, 479), who recovered only 92·8 per cent. of the nitrogen in tryptophan solutions after a 2-hr. digestion with mercuric oxide. The authors found 92·7 per cent. recovery after a total digestion of 35 min., 97 per cent. after 55 min., 99·4 per cent. after 85 min., and 100 per cent. after 115 min. The method described is applicable to the gramicidin-formaldehyde reaction product. A. O. J.

Determination of Silicon in Relatively Non-volatile Organosilicon Compounds. H. Gilman, R. N. Clark, R. E. Wiley, and H. Diehl (*J. Amer. Chem. Soc.*, 1946, **68**, 2728)—Of the availa to methods for the conversion of such compoundsble silicon dioxide, heating with 60 per cent. perchloric acid has been found both rapid and accurate, and preferable to heating with 72 per cent. acid as it reduces carbonisation and produces a smoother reaction. With easily oxidised compounds, a mixture of nitric and perchloric acids can be used.

Procedure (A)—Treat 0·2 g. of the compound in a platinum crucible with 5 ml. of 60 per cent. perchloric acid, and evaporate off the acid by heating on an air-bath over a Meker burner; then ignite to constant weight. Conversion to silica is complete, and the reaction is smooth; a complete determination takes about 3 hr. Condensation of fumes on wood must be avoided, as the impregnated wood then burns vigorously and may explode when heated.

Procedure (B)—Treat the sample as in (A), but use a mixture of 2 ml. of concentrated nitric acid with 5 ml. of 60 per cent. perchloric acid. Heat cautiously as there is a tendency to frothing; after the preliminary evaporation, rinse down the ring of foam near the crucible rim with 3 ml. of perchloric acid, and evaporate again to dryness.

Results—On four samples analysed by method A, a maximum deviation of 1 part in 103 is recorded, results being reproducible to 1 in 130. By method (B) the reproducibility is within 1 in 133, and the accuracy on one sample is approximately 1 in 300. M. E. D.

Determination of the Pyrethrin Content of Dilute Preparations of Pyrethrum Flowers in Oil. G. T. Bray and K. A. Lord (*J. Soc. Chem. Ind.*, 1946, **65**, 382–384)—The authors have investigated the effects of the following factors on the determination of the pyrethrin content of preparations containing less than 0·2 per cent. of total pyrethrins by the Wilcoxon-Holaday method as modified by Martin and Brightwell (*Ibid.*, p. 379): time of saponification; concentration of alkali and time of saponification; degree of dilution of the solution; removal of free acids; and replacement of alcohol by ethylene glycol monoethyl ether as a solvent for sodium hydroxide in the hydrolysis of the pyrethrins. They conclude that (1) for preparations containing 0·2 per cent. (w/v) of total pyrethrins or over, two hours' saponification with *N* alcoholic sodium hydroxide is adequate. (2) For preparations containing less than 0·2 per cent. of total pyrethrins: (*a*) the time of saponification should be 4 hours with *N* alcoholic sodium hydroxide, or 2 hours with 2 *N* alkali, the latter being preferable, (*b*) there is usually no need to remove free acids before saponification, and (*c*) should it be desired to remove the free acids, no solvent should be added when shaking with barium hydroxide, and the aqueous solutions should later be washed with light petroleum. (3) Ethylene glycol monoethyl ether is a satisfactory solvent for use with dilute pyrethrum preparations, and permits a reduction in the time required for saponification. E. M. P.

Quantitative Analysis of Isomeric Cresols and Cresol-Phenol Mixtures by Ultra-violet Absorption Spectra of Vapours. W. W. Robertson, N. Ginsburg, and F. A. Matsen (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 746–750)—Working curves for cresol mixtures were prepared from microphotometer tracings of ultra-violet absorption spectrograms of the vapours of the three pure cresols and a number of synthetic mixtures. The strongest bands in the spectra of the *o*- and *p*-isomers with wavelengths 2744 and 2830A, respectively, are relatively independent; the concentration of either *o*- or *p*-cresol in a mixture can thus be obtained from the density, corrected for background, of the centre of the corresponding band. As the strongest band of *m*-cresol, at 2779A, has a low extinction coefficient and is overlapped by an *ortho* band of almost equal intensity, it is not possible to determine the *meta* concentration by the same direct method. The difference in density between bands of *o*- and *m*-cresols in a single mixture as a function of the meta concentration is used instead.

Owing to the very strong absorption of the *o*- and *p*-bands used for the analysis, samples containing more than 50 per cent. of *ortho*, or 70 per cent. of *para*-cresol cannot be determined directly. The relative concentrations of these isomers can, however, be lowered by addition of a known amount of one of the isomers as a diluent. The mean deviation between the composition of the synthetic cresol mixtures and the composition of these samples as obtained from the working curves is less than 2 per cent.

Working curves for phenol - cresol mixtures based on the phenol band 2637A were prepared as indicated above, and gave a mean deviation of less than 3 per cent. for the phenol content of synthetic mixtures. Samples containing more than 40 per cent. of phenol cannot be analysed directly, but the dilution method already mentioned can be applied.

Using the phenol band 2750A, low concentrations of phenol, probably down to 0·3 per cent., can be determined with a reasonable degree of accuracy. D. A. P.

Inorganic

Photometric Determination of Bismuth in Copper. A. I. Kokorin and I. G. Dermanova (*Zavod. Lab.*, 1946, **12**, 59–63)—Optimum conditions for the determination of bismuth by means of the yellow colour given by ammonium thiocyanate are worked out. As applied to the analysis of copper, it is superior to the potassium iodide method, which requires more separations.

Under similar conditions, the colour intensity is proportional to the bismuth concentration. With increasing amounts of diluted sulphuric acid (1+10) in a total volume of 10 ml. containing 0·06 mg. of bismuth and 0·5 g. of ammonium thiocyanate, the intensity increases with amounts up to 3 ml. of the acid, and then remains constant up to 8 ml.; in 3·5 *N* acid, decomposition occurs. With increasing amounts of ammonium thiocyanate (50 per cent. solution) in 10 ml. containing 3 ml. of diluted sulphuric acid (1+10) and 0·06 mg. of bismuth, the intensity increases with amounts up to 0·9 ml., and then remains constant up to 4 ml. Hydrochloric acid and other chlorides reduce the intensity; 1 ml. of diluted hydrochloric acid (1+1) completely decolorises 10 ml. of solution containing 0·06 mg. of bismuth and 0·5 g. of thiocyanate, but 0·1 ml. has no appreciable effect. Tin introduced as chloride reduces the intensity only because of the chloride present. Iron interferes unless reduced to the ferrous state; for reduction, stannous chloride may be used if comparison is made with standards containing the same amount of reagent. Thus, with 5 mg. of iron, 3 to 4 drops of a 20 per cent. solution of $SnCl_2,2H_2O$ in diluted hydrochloric acid (1+1) may be used satisfactorily. Antimony gives no colour, and when less than 0·5 mg. in amount, has no effect on the bismuth colour. Lead gives lead sulphate, which must be filtered off. Separation from copper may be effected by double precipitation with aqueous ammonia in the presence of aluminium salts. The error in the determination of bismuth in the range 0·0005 to 0·003 per cent. with a 5-g. sample does not exceed about 1 in 10.

Procedure—Dissolve 5 g. of copper in 60 ml. of diluted nitric acid (1+1), boil to remove oxides of nitrogen, dilute with water to about 200 ml., cool, introduce 2 ml. of 10 per cent. aluminium nitrate solution, and, avoiding a large excess, add 25 per cent. aqueous ammonia solution (about 28 ml. will be required) until all the copper is in the form of the ammonia complex. Leave cold for 15 to 20 min., filter through a filter funnel (diameter of pores, 100–110 μ), wash with cold water containing 1·5 to 2 ml. of concentrated aqueous ammonia in 100 ml., dissolve the precipitate in 4·5 ml. of hot diluted sulphuric acid (1+2), wash with hot water, and precipitate as before. Again dissolve in diluted sulphuric acid (4·5 ml. of 1+2 acid), but collect the filtrate in a 15-ml. graduated flask (or burette). If necessary, refilter. Cool the solution, the volume of which with the washings should be about 13 ml., add 2 ml. of 50 per cent. ammonium thiocyanate solution, and four drops of stannous chloride solution in hydrochloric acid (1+1), close the flask, and shake to decolorise the ferric thiocyanate complex. Measure the intensity of colour by means of a compensation photo-colorimeter, and determine the bismuth concentration from calibration curves.

G. S. S.

Quantitative Method for Estimation of the Arsenoso Group. C. K. Banks and J. A. Sultzaberger (*J. Amer. Chem. Soc.*, 1947, **69**, 1–4)—Arsenoso compounds prepared by the usual synthetic methods are frequently of dubious homogeneity, being contaminated with the corresponding arsonic acid, the de-arsenated organic residue, arsenic trioxide, or mixtures of these substances. The determination of total arsenic by wet combustion will detect gross contamination, but considerable amounts of impurities may be present without changing the total arsenic content by an amount greatly different from the tolerance limits usually prescribed for such analytical procedures. Examination of the literature showed that reduction reactions are not quantitative, but that it might be possible to develop a quantitative oxidation method. Apparently, the general applicability of iodine titration of arsenoso groups has not been thoroughly investigated.

Theoretically, an arsenoso compound or an arsonous acid should react with iodine according to the equation

$$R\text{-}As(OH)_2+I_2+H_2O \rightleftharpoons R\text{-}AsO_3H_2+2HI.$$

Of the common impurities, only arsenic trioxide might be expected to react with iodine. Also, iodination of the organic nucleus of the arsonic acid or de-arsenated organic residue might interfere with the stoicheiometric reaction. Although arsines and arseno compounds will also react with iodine, the first cannot exist in presence of arsenoso compounds, and the second has never been known to occur as an impurity, although the presence of small amounts of arsenoso compounds in arseno compounds is frequent.

Highly purified samples of 3-amino-4-hydroxybenzenearsonous acid and 4-[2′:4′-diamino-6′-triazinylamino]-benzenearsonous acid hydrate were used to test the stoicheiometric behaviour of the arsenoso-iodine reaction, and these, in acidified aqueous solution, were titrated with standard iodine solution. The reaction was found to be both stoicheiometric and of adequate precision. The effect of interfering substances was also investigated. From previous unpublished work it was known that, in the presence of an excess of iodide ion, most benzenearsonic acids do not reduce above *p*H 1·1, and none of those examined reduced above *p*H 2. On the other hand, arsenic acid showed evidence of reduction at all *p*H values below 4·8. The reaction of iodine with varying amounts of arsonous acid, arsonic acid, and arsenic trioxide between *p*H 2 and 6·8 was therefore investigated. It was found that the reaction between the arsonous acid and iodine remained stoicheiometric between these *p*H values, and that the presence of the arsonic acid had no effect on the titration. Arsenic trioxide interfered above *p*H 5 when it constituted more than 4 per cent. of the arsonous acid, but between *p*H 2 and 3 the reaction was within the analytical limits with as much as 8 per cent. of arsenic trioxide present. With 11 per cent. of arsenic trioxide, the end-point of the reaction was not definite, and the iodine consumption was not stoicheiometric for the arsonous acid, and did not approach the value required for the combined organic and inorganic tervalent arsenic. The amount of arsenic trioxide that failed to interfere with the stoicheiometric properties of the arsonous acid and iodine reaction appeared to be a function of the amount of arsonous acid present.

To determine why small amounts of arsenic trioxide failed to influence the titration appreciably, the effect of each constituent present in the reaction was investigated. Variation of *p*H alone, although influencing the arsenic trioxide and iodine reaction, did not explain the exclusion of inorganic arsenic from the titration. Similarly, the presence of the amount of iodide ion equivalent to that produced during the titration of about 0·1 g. of arsenoso compound failed to influence the arsenite reaction. The amount of *p*-arsanilic acid that would be produced under such conditions did significantly alter the reaction, and a combination of *p*-arsanilic acid and iodide ion sufficed to reduce the iodine titre of 1·5 mg. of arsenic trioxide from 0·570 to 0·008 ml. Similarly, the titre for 12 mg. of arsenic trioxide was reduced from 4·560 to 0·024 ml. Since the titration of 0·1 g. of most arsenoso compounds or arsonous acid involves about 20 ml. of such an iodine solution, 0·008 ml. would be insignificant and 0·024 ml. barely noticeable. This phenomenon was noted with over twenty benzenearsonic acids besides *p*-arsanilic acid.

A number of arsenoso compounds and arsonous acids were titrated under what appeared to be optimum conditions, and the values found all compared favourably with those obtained by combustion methods and with those required by theory. The following method was found satisfactory for the titration of arsenoso compounds and arsonous acids.

Procedure—Dissolve the sample (0·15 to 0·20 g.) in 40 ml. of water and add, if necessary for complete solution, a few drops of hydrochloric acid. When the sample is not soluble under these conditions, dissolve it in 15 ml. of propylene glycol by warming gently, and dilute the solution with water. Adjust the *p*H to about 3·5 and titrate with 0·05 *N* iodine, using starch as indicator.

It is found advisable in practice to determine both the tervalent organic and total arsenic values. As an arbitrary standard, if both values are within ±0·1 per cent. of theory and each other, the compound is considered to be pure within the limits of the analysis. If theory and the two assays agree within 0·3 per cent., the compound is generally considered sufficiently pure for pharmacological purposes (over 99 per cent.). Occasionally, samples are obtained for which the two assays agree to within ±0·1 per cent., but do not agree with the theoretical value either for the arsonous acid or the arsenoso compound. Although this may be due to the presence of non-arsenical organic or inorganic compounds, it is frequently caused by incomplete dehydration of the forms $RAsO_3H_4$ or $RAsO_2H_2$.

The iodine solution should be standardised against Bureau of Standards arsenic trioxide and acidified standard sodium thiosulphate solution. Iodine solutions may acquire a small amount of iodate on standing, and although this is not important in assay procedures where the iodine solution is standardised under the conditions of the assay, it is a source of appreciable error in this determination. In acid solution the iodate is available, but it does not react under the conditions of the standardisation against arsenic trioxide. Iodine solutions showing detectable amounts of iodate should not be used to titrate arsonous acids. The inhibiting effect of *p*-arsanilic acid on titration of the arsenite ion with iodine is being investigated. A. O. J.

Rapid Method of Determining Sulphate Impurities in Cryolite. J. V. Karjakin and V. I. Muraschova (*Zavod. Lab.*, 1946, **12**, 284–286)—Sulphate-contamination of manufactured cryolite is apparently an adsorption effect. Double precipitation of the hydrochloric acid solution with aqueous ammonia and solid sodium chloride gives almost complete desorption, and sulphate may then be determined by boiling the solution with barium chromate suspension in hydrochloric acid, neutralising with aqueous ammonia, and titrating the chromate in solution equivalent to the barium sulphate precipitated (Andrews, *Amer. Chem. J.*, 1889, **11**, 567; Komarowsky, *Chem.-Ztg.*, 1907, **31**, 498). The method is claimed to be more rapid than any previously described (it takes from 50 min. to 1 hour) and to have a maximum relative error of ±10 per cent. The results compare well with those obtained by Schrenk and Ode's method (*Ind. Eng. Chem., Anal. Ed.*, 1929, **1**, 206) based on the removal of fluorine by evaporation with hydrochloric and boric acids.

Procedure—Dissolve 1 g. of cryolite in 25 ml. of diluted hydrochloric acid (1+2) by heating in a 100-ml. conical flask under cover. Make just alkaline to methyl red with diluted aqueous ammonia (1+1), add 3 g. of dry sodium chloride, heat to boiling, allow to stand for a short time, and filter through a 9-cm. paper, washing flask and filter once with sodium chloride solution (1·5 per cent.). Return the filter paper and precipitate to the original flask, break up the paper, add 20 ml. of diluted hydrochloric acid (1+2), and heat to dissolve the cryolite and to pulp the paper. Repeat the precipitation with aqueous ammonia and sodium chloride, filter, wash three times with the sodium chloride solution, combine the filtrates from the first and second precipitations, make just acid to methyl red with hydrochloric acid, heat to boiling, and add 2 ml. of barium chromate suspension (obtained by mixing hot solutions of 19·44 g. of potassium chromate in 1 litre of water and 24·44 g. of $BaCl_2.2H_2O$ in 1 litre of water) and sufficient diluted hydrochloric acid (1+2) to ensure solution of the excess barium chromate. Boil the mixture, containing insoluble barium sulphate, for 10 to 15 min., cool under the tap, make just alkaline with aqueous ammonia (yellow to methyl red), transfer the solution to a 100-ml. measuring flask, and make up to volume. Mix, filter through a dry paper, and pipette 25 ml. of the filtrate into a 100-ml. flask already containing a mixture of 10 ml. of potassium iodide (10 per cent.) and 10 ml. of diluted hydrochloric acid (1+1) that has been allowed to stand for 5 min., and has been decolorised, if necessary, by means of a drop or two of thiosulphate. Leave for 5 min. in the dark, and titrate the iodine liberated, using starch as indicator, with 0·1 *N* thiosulphate, the titre of which has been established on cryolite containing a known percentage of sulphate.

G. S. S.

Gas Analysis

Traces of Oxygen in Hydrogen or Nitrogen. H. A. Liebhafsky and E. H. Winslow (*J. Amer. Chem. Soc.*, 1946, **68**, 2734–2735)—By the method of Winslow and Liebhafsky (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 565), which is sensitive to 0·0001 per cent. of oxygen by volume, the efficiency of hot copper in removing oxygen from gas samples has been tested. Passage of hydrogen over copper turnings at 600° C. in a fused-silica tube removes all the oxygen, whilst 0·0017 per cent. by volume remains if a temperature of 230° C. is used.

The removal of oxygen from nitrogen is facilitated by adding hydrogen, the end-product then being water and not copper oxides. At a temperature of about 400° C., introduction of 25 per cent. of hydrogen results in a purified gas retaining 0·0009 per cent. of oxygen, while with only 3 per cent. of hydrogen the purification is to 0·0002 per cent. of oxygen.

"Oxygen-free" gases are contaminated by oxygen in storage. Thus, hydrogen was found to pick up 0·0026 per cent. of oxygen on passing through 10 ft. of rubber tubing, and nitrogen 0·0035 per cent. of oxygen in a "sealed" steel system. M. E. D.

Microchemical

Micro-determination of Pentoses by the Bisulphite Method. V. Heines (*Arch. Biochem.*, 1946, **11**, 531–535)—The micro-determination of *d*-xylose and *d*-ribose by conversion to furfural has been studied by two methods, one involving distillation of the furfural into a sodium bisulphite solution, and the other depending on the titration of the furfural without preliminary distillation. The first method can be used for the determination of 10 to 30 mg. of pentose, and the second for 500 µg. to 5 mg.

Reagents—Dissolve 0·950 g. of anhydrous sodium metabisulphite in water to form 1 litre of 0·02 *N* solution. Standardise 0·01 *N* iodine against 0·01 *N* thiosulphate, using starch as indicator; prepare the iodine solution fresh before each series of experiments, and check it frequently. Prepare aniline acetate test paper from freshly distilled aniline, free from furfural.

Pentose determination with distillation of furfural—Pipette 3 ml. of pentose solution containing 10 to 30 mg. of the sugar into a test tube and add 10 ml. of 85 per cent. phosphoric acid solution. Heat in an electrically controlled asbestos mantle to 125° C., and then connect to a steam generator and steam-distil into an ice-cooled receiver. Allow the temperature of the mantle to reach 175° C., but not higher, and continue the distillation until the distillate fails to show a red colour with aniline acetate paper. Dilute to a definite volume, usually 200 ml., and treat aliquot portions with an excess of 0·02 *N* bisulphite to form the furfural-bisulphite addition compound. Oxidise the excess of bisulphite with 0·01 *N* iodine, using starch as indicator. After the first end-point, discharge the blue colour with a few ml. of saturated sodium bicarbonate solution, and titrate the freed bisulphite to the reappearance of the blue colour. If a blank determination is run, a check may be made on the first end-point, under exactly the same conditions of temperature, *p*H, and concentration.

Pentose determination without distillation of the furfural—Place 1 ml. of a solution containing from 500 µg. to 5 mg. of pentose in a flask connected to a small reflux condenser, and add 2 ml. of 85 per cent. phosphoric acid. Maintain the mantle at about 130° C. for 10 min., allow to cool, and dilute to 100 ml. Take aliquot portions, chill, and neutralise to methyl orange. Treat with an excess of 0·01 *N* bisulphite, and allow to stand for about 2 hours at room temperature to ensure complete formation of the furfural-bisulphite compound. Titrate with 0·005 N iodine in a burette graduated in 0·05-ml. divisions. Record the first end-point.

Calculation—A sample of 15 mg. of *d*-xylose converted to furfural by heating with 85 per cent. phosphoric acid was steam-distilled into 25 ml. of 0·02 N sodium metabisulphite solution. On titrating with 0·0096 N iodine, the second end-point gave 10·40 ml. of iodine used to react with the freed bisulphite: then, ml. of iodine used × normality of iodine × 75 = mg. of pentose. Hence, 10·40 ml. of iodine × 0·0096 N iodine × 75 = 7·47 mg. of *d*-xylose. Using the factor 2, the recovery in milligrams equals 14·94, representing a 99·6 per cent. recovery of pentose.

Data showing that determinations, without distillation of the furfural, gave an average of 95 per cent. recovery of sugar from samples containing 500 µg. to 5 mg. of *d*-xylose or d-ribose per millilitre are recorded. E. M. P.

Physical Methods, Apparatus, etc.

Polarographic Analysis of Brass Plating Baths and Brass Coatings. D. A. Viachirev (*Zavod. Lab.*, 1946, **12**, 276–278)—Since only 0·5 ml. of the electrolyte is required, complete decomposition of the cyanides may be effected in a few minutes, and by using a small Kjeldahl flask with a 25-ml. mark on the neck, the decomposition flask may be used as a measuring flask. *Procedure*—Decompose 0·5 ml. of the cyanide electrolyte, in a small Kjeldahl flask having a narrowed neck with a 25-ml. mark, by heating with a mixture of concentrated sulphuric and nitric acids. The flask is placed in an inclined position on a sand-bath. After destruction of the cyanide complexes (about 5 to 10 min.), cool in running water, add 2 to 5 ml. of water, and carefully neutralise with aqueous ammonia, bringing copper and zinc into solution and precipitating iron. Add 1 ml. of freshly-prepared gelatin solution, cool, and make up to the mark with 25 per cent. aqueous ammonia solution. Leave to stand and decant, or filter directly into a polarographic cell, and take the copper and zinc waves. Calculate the contents of copper and zinc from calibration curves.

For coatings, make the sample the anode and a platinum spiral the cathode in a mixture of 30 ml. of 25 per cent. aqueous ammonia solution and 2 ml. of saturated ammonium nitrate solution, electrolyse for 10 to 15 min. with a current density of 2 to 3 amp. per sq.dm., remove the electrodes, re-insert the platinum spiral, and stir to dissolve the deposit. Take polarograms for copper and zinc. Alternatively, dissolve the coating on the sample, without electrolysis, as described by Tyler and Brown (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 521). The accuracy is 2 to 3 parts in 100 with the electrolyte and 4 to 5 parts in 100 with the coating. G. S. S.

Polarographic Determination of Lead in Lead Slags. S. J. Fainberg and E. M. Tal (*Zavod. Lab.* 1946, **12**, 265–268)—*Procedure*—To 0·1 g. of slag in a 50-ml. conical flask, add 0·1 g. of ammonium or sodium fluoride and about 1 ml. of water, carefully mix, add 5 ml. of concentrated hydrochloric acid, heat gently to effect complete decomposition, and evaporate nearly to dryness. Cool, add 10 ml. of diluted hydrochloric acid (1+1), boil to dissolve the salts, cool somewhat, and pour into a 25-ml. measuring flask, rinsing with a few ml. of diluted hydrochloric acid (1+1). Reduce ferric iron by heating the solution to complete decolorisation with about 1 g. of reduced iron (1 to 2 min.) or a 2 to 3-cm. iron nail (20 to 25 min.), cool in running water, dilute to the mark with diluted hydrochloric acid (1+1) adding 5 to 6 drops of 0·25 per cent. glue solution, and take a polarogram for lead. Passage of hydrogen is unnecessary. A complete determination takes only 20 to 25 min. To standardise, place in three separate measuring flasks (25-ml. capacity) 2, 3, and 5 mg. of lead as lead nitrate solution, add 30 mg. of iron as ferric chloride, evaporate to dryness, add 10 ml. of diluted hydrochloric acid (1+1), and then reduce with iron and proceed as above. The method was tested on samples containing 1·5 to 2·3 per cent. of lead. Errors do not exceed about 1 in 30. Tungsten, thallium, tin, and arsenic, which would interfere, are not encountered in lead slags. Bismuth and copper in small amounts have no effect. G. S. S.

Polarographic Analysis of Nitrite and of Nitrite - Nitrate Mixtures. B. Keilin and J. W. Otvos (*J. Amer. Chem. Soc.*, 1946, **68**, 2665–2668)—In the presence of uranyl ions and dilute hydrochloric acid, nitrite and nitrate ions are both reduced at approximately −0·9 v. (*versus* the Saturated Calomel Electrode). The diffusion current is proportional to the nitrite concentration when the ratio of uranyl ion to nitrite is greater than unity. Under these conditions, the reduction of nitrite involves three electrons, indicating a reduction to nitrogen. Analysis of the wave shows that the reduction is irreversible. A solution can be analysed for both nitrate and nitrite ions in two polarographic experiments. The diffusion current due to the two constituents in the original solution is first measured. The nitrite in a second sample is oxidised to nitrate by hydrogen peroxide in acid solution, the excess of peroxide destroyed catalytically by manganese dioxide in alkaline solution, and the diffusion current measured.

Procedure—*Nitrite*—Prepare two stock solutions that are 0·2 M with respect to potassium chloride and 0·02 M with respect to hydrochloric acid, the first being $4 \times 10^{-4}\,M$, and the second $1 \times 10^{-4}\,M$ with respect to uranyl acetate. Measure into a 50-ml. flask a volume of nitrite solution, such that the final nitrite concentration lies between 5×10^{-5} and $5 \times 10^{-3}\,M$, and add the 25 ml. of either the first stock solution for nitrite concentrations above $1 \times 10^{-4}\,M$, or the second stock solution for lower concentrations. Dilute to 50 ml., transfer an aliquot portion to a polarographic cell, pass nitrogen gas to remove dissolved oxygen, and measure the diffusion current at a potential of −1·2 v. against the Saturated Calomel Electrode. Carry out a blank determination on the stock solution, and from the corrected diffusion current, calculate the nitrite concentration by reference to a calibration curve.

Nitrite and Nitrate—Divide the solution to be analysed into two equal portions. Examine the first as above, and obtain the diffusion current due to nitrite and nitrate. Neutralise the second portion with 2 *N* hydrochloric acid, add an excess of 5 drops, and 1 ml. of 30 per cent. hydrogen peroxide. Allow to stand at room temperature for 30 min., add 8 drops of 2 *N* sodium hydroxide and a small quantity of manganese dioxide. After the evolution of gas has ceased, decant the solution quantitatively to a 50-ml. flask, add 3 drops of 2 *N* hydrochloric acid, and 25 ml. of the appropriate uranyl acetate solution, dilute to 50 ml., and measure the diffusion current for an aliquot portion. The nitrite and nitrate concentration can be calculated from a standard curve, or from equations (1) and (2):

$$C_1 = \frac{i'_d - i_d}{6{\cdot}35\, m^{2/3}\, t^{1/6}} \qquad \cdots \quad \cdots \quad (1)$$

$$C_2 = \frac{i'_d}{13{\cdot}8\, m^{2/3}\, t^{1/6}} - C_1 \qquad \cdots \quad (2)$$

where C_1 and C_2 are the concentrations of nitrite and nitrate, respectively; i_d and i'_d are the corrected diffusion currents for the original and the oxidised solutions, respectively; m is the weight of mercury flowing in mg. per sec., and t is the drop-time in seconds.

Interferences are the same as those described in the method for determining nitrate (ANALYST, 1945, **70**, 101). Large amounts of sulphate ions also reduce the diffusion current. J. G. W.

Electron Diffraction and Electron Microscopic Study of Oxide Films formed on Metals and Alloys at Moderate Temperatures. R. T. Phelps, E. A. Gulbransen, and J. W. Hickman (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 391–400)—The physical and chemical structure of oxide films formed under known conditions on chromium, cobalt, copper, iron, molybdenum, nickel, aluminium, niobium, and tungsten have been studied by reflection and transmission methods of electron diffraction, and by electron microscopy. Electron diffraction reflection patterns of the polished metal specimen are taken in a camera furnace (E. A. Gulbransen, *J. Applied Phys.*, 1945, **16**, 718–724) before and after oxidation, and after cooling *in vacuo*. The specimen is then cut in half and photomicrographs × 100 and × 1000 are taken of one piece, and a thin film is formed on the surface of the other from a 0·01 per cent. solution of nitrocellulose in amyl acetate. The film is cut into 1 mm. square sections with a needle point, and the remainder of the specimen is painted with a protective coating. The film is removed electrolytically or chemically, washed, mounted, and examined by electron microscopy and transmission diffraction.

The electrolytic cell used for the removal of the films is modified from that of Evans and Stockdale (*J. Chem. Soc.*, 1929, 2651). A U-shaped cell fitted with three side arms is divided by a fritted-glass filter. The electrolyte is boiled saturated potassium chloride solution, and the atmosphere above the cell is pure hydrogen. The oxidised metal is the anode and the cathode is the same metal, or platinum. A current source of 110 v. D.C. is controlled by a potentiometer-rheostat, and a second rheostat in series with the electrodes. A current of 10 m.amp. is increased gradually to 60 m.amp. if the film is not loosened within an hour.

The experimental values for lattice spacings are compared with X-ray data, and are used to calculate the lattice parameter for cubic-type oxides. Electron reflection yields parameters 0·7 per cent. higher than accepted X-ray values, and the transmission method gives values that average 0·2 per cent. low. The electron and light micrographs show that the films consist of oxide crystals 100 to 2500 A. in size; they are about 10^{-3} to 10^{-5} of the linear dimension of the metal crystal grain. The crystal size is found to be a function of time and temperature of oxidation.

The oxide films are largely non-uniform, as they consist of thicker and thinner sections; this indicates a multi-layer film of oxide crystals. Nucleation thus occurs in contact not only with the initial thin oxide layer but also with other oxide crystals. Overlapping of one crystal on another often occurs. The oxides formed on tungsten, niobium, and chromium give the smallest average size, whilst the largest crystals occur during oxidation of copper, iron, molybdenum, nickel, and cobalt. The more protective metals thus appear to have the more uniform films.

Electron micrographs, light micrographs, and electron diffraction patterns for the nine metals investigated are reproduced in the paper.

G. A. B.

Determination of Tetraethyl Lead in Gasoline by X-Ray Absorption. M. V. Sullivan and H. Friedman (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 304–306)—The X-ray absorption technique described by Aborn and Brown (*Ibid.*, 1929, **1**, 26–27) is limited in sensitivity by the use of an ionisation chamber to detect the radiation. This paper gives details of the use of a Geiger counter as detector, and describes how this modified method can be used to detect, for example, 0·005 ml. of tetraethyl lead per gallon of gasoline. The accuracy is comparable with that obtained by chemical methods, yet this method takes only about 5 min. to carry out and is potentially semi-automatic.

Lead has a high absorption coefficient for nearly all X-ray wavelengths and therefore no attempt was made to select a characteristic absorption edge. Using an absorption cell length of 15 to 25 cm., the optimum conditions were obtained with an X-ray tube (molybdenum target and line focus) operated at 17 kv. The Geiger counter used (see Friedman, *Electronics*, 1945, **18**, 132–7), had a quantum efficiency of 80 per cent. for the radiation employed, and was connected to an amplifier capable of passing 25,000 random counts per sec. coupled to a scale of 32, the output being registered by a mechanical impulse counter.

Development of this method for the detection of metals in general by making use of absorption edges may open up new fields for this method of analysis in fuel and oil research, in the study of "break in" and "break down," and in the selective observation of rates of wear of different metallic sections such as shafts and bearings. E. G. S.

Preparation of Standard Powders for Reference in Particle-size Measurement. E. L. Gooden and R. L. Updike, jun. (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 802)—Two economic procedures for preparing standard powders are given. (A) Glass beads used for coating projection screens contain a considerable percentage of material, easily separated by sieves, that is close to 100 microns in diameter. Non-spherical particles are removed from this material by passing it through a slightly oversize sieve with little or no agitation. (B) A grade of glass wool with a cross-sectional diameter about 8 μ. is cut into short lengths with shears or a print trimmer and ground in a hammer mill. Final calibration of the powder is done with a microscope. The acicular powder has a surface mean particle diameter about 11 μ., and the spherical powder about 110 μ.

G. A. B.

Qualitative Method for Detecting Surface-Active Agents. L. F. Hoyt (*J. Amer. Oil Chem. Soc.*, 1947, **24**, 54)—The method is based on the solubilisation in aqueous solution of certain oil soluble dyes, particularly Brilliant Oil Blue BMA.

Preparation of sample—(1) Dry materials—Grind to a fine powder; digest 10 g. with 50 ml. of hot, 50 per cent. alcohol and filter through paper with suction. Evaporate the residue to dryness; digest this residue with 3 ml. of distilled water by warming in a steam-bath, and filter the water extract through a wet filter in a 1-inch funnel. (2) Liquids or pastes—Extract 10 g. with three successive portions of benzene, chloroform, or trichloroethylene; combine the extracts, filter, and evaporate to dryness. Treat the residue with 3 ml. of water as in (1).

Procedure—Add 0·02 g. of a finely ground mixture of 2 parts of National Brilliant Oil Blue BMA with 98 parts of sodium chloride, prepared by ball-milling, to the water extract from (1) or (2). Shake and allow to stand for an hour or more. Compare with a "blank" of 0·02 g. of the dye reagent in 2 ml. of water. Half a milligram, often less, of the organic component of a surface active agent will produce a blue solution, the blank test showing only a faint red-purple tinge.

Oil Red O or Oil Yellow AB may be used as dyes in the test, but are not universally applicable. With the blue dye, 48 agents gave positive results. Alframine DCA and a cationic agent, Retravon, gave negative results. Soap solubilises all three dyes and must be eliminated by acidifying, filtering, and neutralising the filtrate before the test is made.

Reviews

CURRENT WATERWORKS PRACTICE: A PRACTICAL TREATISE ON THE PROVISION OF WATER SUPPLIES FOR URBAN AND RURAL COMMUNITIES. By W. H. MAXWELL, A.M.Inst.C.E. Pp. viii + 254. London: B. T. Batsford, Ltd. 1946. Price 18s. net.

During the past ten or fifteen years, no more pressing subject to the sanitarian than that of water supply has occupied public attention and, despite the impact of war with all its preoccupations and disturbing effects, the passing of the Rural Water Supplies and Sewerage Act, 1944, and the Water Act, 1945, bears testimony to the will of Parliament to deal with a truly national problem. These enactments have focussed attention on the implications of hydrology in its widest interpretation and it is more than ever realised—with the ever-increasing and co-ordinated knowledge and application of the principles underlying conservation, storage, distribution and sterilisation of potable water—how ultimate success depends upon the co-operation of the engineer, the practical geologist, the chemist and the biologist.

The appearance, therefore, of a limited treatise, embodying a condensed survey of the many mechanical, structural and technical branches of the subject—from initial hydrogeological considerations of the proposed supply to its final conditioning for consumption—

written by a reputable civil engineer of many years' *practical* experience in waterworks' design and equipment, is timely and should appeal to the chemist entrusted with joint responsibilities governing the provision and scientific control of pure and wholesome drinking water. At the same time, he must not expect to find herein much beyond what may help him towards a clearer appreciation and more complete understanding of the work of his engineering and other colleagues.

About the substance, however, of what has been included in a wide review of modern practice, there is evidence of a careful discrimination and selection, although one gets the impression that the author, in his desire to incorporate a digest of all that is germane to the subject, has, in certain respects, not allowed himself sufficient elbow room. This is not to be interpreted as a call for any drastic expurgation of the text but rather the desirability of a re-editing, in some future revision, of certain of the contents which, in other respects, are concise and eminently readable.

Naturally, the mechanical and constructional aspects of the reservoir site, plant, and auxiliaries and so forth, receive major attention and, generally speaking, the book is well abreast of the latest engineering developments and makes a useful contribution to waterworks' literature and should thus prove of service to practising water engineers and others engaged in the industry, being clearly outlined and ably written. The value of the text, in this section of the review, is much enhanced by inclusion of many helpful references to existing undertakings which illustrate good practice. In this relation, for example, the use of concrete is fully discussed and much up-to-date information is summarised. The subject of distribution, however, is but briefly dealt with, and it would appear more could appropriately have been included, particularly about the design of such systems. Nor can one resist the impression that more prominence might well have been given to the conservation of water from other than underground sources.

Methods of treatment and purification—including all-too-brief reference to such recent developments as "break-point" chlorination, "Calgon" stabilisation, ion-exchange systems, anti-corrosion measures and so forth—in which the chemist is more intimately concerned, are confined almost entirely to two chapters (XI and XIII). The second of these is, in effect, an abbreviated *olla podrida*, ranging from a timely caution on engineering structures *vis-a-vis* modern methods of destruction to flocculation of fine suspensions by supersonic vibrations! Incidentally, their significance in such a compendium would have been materially enriched by an adequate extension of footnote references to current literature, enabling the reader to follow up the theme in more detail. It is in no spirit of carping criticism to suggest that the various phases which are within the ambit of the chemist should have been accorded a more orderly presentation *in one chapter or section* and the ritual of purification principles adopted, from source to consumer's tap—pre-treatment, storage, flocculation, sedimentation, filtration, correction, and so on—assembled, however much condensed, in logical sequence.

These minor defects notwithstanding, there is so much sound and finely illustrated material set out that, as a book of reference, it can be commended to the chemist who is primarily concerned with water supply, as an intelligible and useful volume to retain in his library.

S. Ernest Melling

The Nation's Food: A Survey of Scientific Data. Edited for the Society of Chemical Industry by A. L. Bacharach, M.A., F.R.I.C., and T. Rendle, F.R.I.C. Pp. 349. London: Society of Chemical Industry. 1946. Price 18s. net.

At the outbreak of war the Nutrition Panel of the Society of Chemical Industry decided to concentrate its activities on those best calculated to be of the greatest utility under the conditions prevailing. This policy led to a series of meetings, held between the years 1940 and 1943, at which papers were contributed on "the nation's food." Each paper covered a separate and specialised field of knowledge and was written by an authority on the subject under review. The authors numbered rather more than thirty, and included amongst the better known were Lampitt, Bacharach, Kent-Jones, Kon, Mamie Oliver and Harriette Chick. This series of papers, edited by Bacharach and Rendle, have been brought together to constitute the book under review.

The foods dealt with included eggs, potatoes, vegetables, cereals, meat, fish and milk. The first article of diet considered was the egg, and the egg, as a food, for instance, was studied from three angles, namely, the biological, chemical, and nutritional. In addition, two chapters were devoted to the preservation of, and the effect of cooking upon, eggs. It may be readily appreciated that, from the chemist's and dietician's point of view, the information about the egg is almost exhaustive. In other chapters, other foods are dealt with in a similarly thorough manner.

Some of the chapters provide material of more than ordinary interest and should make a special appeal to a wide variety of readers. These include, for example, the one that gives an account of the biology of the sea fisheries.

An endeavour is generally made by a reviewer to indicate the class of reader to whom a volume should be of particular use. In dealing with this book a difficulty arises because it contains so much information that it should appeal to anyone who takes a scientific interest in food. Not only does it present a survey of knowledge concerning certain foods but it gives an invaluable collection of analytical results. The student, too, has in this volume information respecting the chemical, physical, and physiological properties of many foods that it would be difficult, or even impossible, for him to obtain elsewhere.

The only regret, perhaps, is that more subjects have not been dealt with, but the Editors express the hope that other foods will be considered in the near future, and presumably therefore, a further volume will be published. May this be soon! F. W. F. ARNAUD

JULY, 1947 Vol. 72, No. 856

THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

PHYSICAL METHODS GROUP

THE Ninth Ordinary Meeting of the Physical Methods Group was held on Tuesday, February 11th, 1947, in the rooms of the Chemical Society, Burlington House, London, W.1., with the Chairman of the Group, Mr. R. C. Chirnside, F.R.I.C., presiding. The subject of the meeting was Fluorimetric Analysis, and the following papers were presented and discussed: "Apparatus Design for Fluorescence Measurements," by E. J. Bowen, M.A., F.R.S.; "Notes on Fluorescence Quenching," by the same author; "Some Applications of Fluorimetry in Vitamin Analysis," by E. Kodicek, Ph.D., M.D.; "The Use of Fluorimetric Analysis in the Study of Pterins," by Delia M. Simpson, M.A., Ph.D.

The Tenth Ordinary Meeting of the Group was held on Friday, May 2nd, 1947, at King's College, Newcastle-on-Tyne. Mr. Chirnside was again in the chair and about eighty members and visitors were present. The following papers on Physical Methods of Gas Analysis were presented and discussed: "Gas Analysis at Low Pressures," by C. E. Ransley, M.Sc., Ph.D.; "The Analysis of Hydrocarbon Gases by Low-Temperature Distillation," by J. H. D. Hooper, B.Sc., A.R.I.C.; "A New Apparatus for Gas Analysis by the Soap Film Method," by W. J. Gooderham, B.Sc., A.R.C.S., F.R.I.C., M.Inst.Gas E., who also demonstrated his apparatus.

POLAROGRAPHIC DISCUSSION PANEL

The first meeting of the panel was held, under the chairmanship of Dr. W. Cule Davies, in the Physical Chemical Lecture Theatre, Imperial College of Science and Technology, London, S.W.7, on Friday afternoon, April 25th, 1947. The attendance was 39 members and visitors and discussions followed contributions by Dr. G. Jessop on "Notes on Polarographic Technique," by Dr. W. Cule Davies on "Remarks on Polarographs" and by Dr. E. R. Roberts on "The Analysis of Brass Plating."

Dr. Jessop dealt first with the instrumental aspects, in particular the measurement of current and potential and the various ways of determining wave height. He discussed such effects as arise from the ohmic resistance of the circuit and from variations in the anode potential, instancing the peculiar wave forms sometimes encountered when sodium sulphite is used in conjunction with a mercury pool anode.

Dr. Cule Davies, discussing desirable features to be incorporated in a polarograph, considered that means should be provided for the accurate measurement of drop-rate, current and voltage, and that damping, if used, should alter neither the magnitude of the current nor the observed half-wave potential. Published accounts of polarographic work should give the characteristics of the dropping electrode, wave heights being expressed in units of current.

Dr. Roberts pointed out that in the bonding of rubber to cast iron the latter must be brass-plated and described polarographic methods of control of the plating, by means of which six bath samples could be analysed within half an hour, while plating could be stripped and examined similarly. De-oxygenation by sodium sulphite gave peculiar results for zinc, but sulphur dioxide was both rapid and effective. A simple cell incorporating an external reference electrode was developed for routine use and enabled results to be obtained by measuring the current at three predetermined potentials.

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Agricultural Research Institute
New Delhi

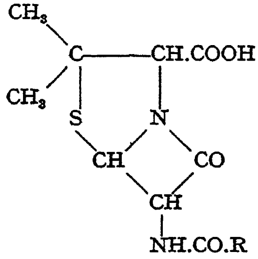
Chemistry of Penicillin

By F. A. ROBINSON

(Read at the meeting of the Society on May 7th, 1947)

THE constitution of penicillin was elucidated as the result of collaborative research carried out in many laboratories in Great Britain and the U.S.A. during 1943–45.[1] In the course of this work, it was discovered that several different penicillins exist. These vary according to the strain of *Penicillium notatum* or *P. chrysogenum* used and with the cultural conditions. Indeed, the proportion of certain penicillins in a mixture can be increased by addition to the medium of certain "precursors" which the mould is apparently able to assimilate and incorporate into the penicillin molecule.

All the penicillins have the common basic structure



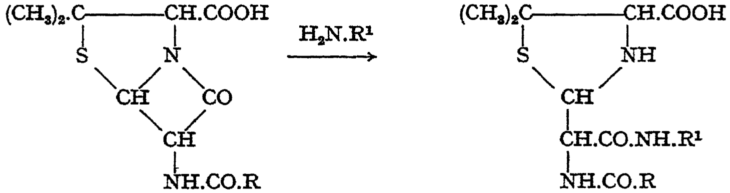
in which the group R varies from one penicillin to another. In the original penicillin produced by Sir Howard Florey and Dr. E. Chain and their colleagues at Oxford, R for the major constituent is a Δ^2-pentenyl group. This species of penicillin was originally termed penicillin F, in order to identify it with the penicillin discovered by Fleming and to distinguish it from another antibiotic produced by *P. notatum*, which was originally called penicillin A and later notatin; it has a structure entirely different from that of the penicillins. The term penicillin F is still used in the U.S.A., but in this country this form is now generally known as penicillin I. Mild methods of reduction convert penicillin I into dihydro penicillin I, in which R is an amyl group, and the activity is apparently undiminished or even slightly increased by the hydrogenation. In America the penicillin originally isolated was not penicillin F, but a different penicillin with approximately the same activity. It was considerably easier to isolate and more stable than penicillin F and was called penicillin G by the Americans; in this country it is known as penicillin II. In this instance the group R is a benzyl group, and the presence of this form in the original American penicillin was associated with the use of a different strain of *P. notatum* and with addition to the medium of corn steep liquor, which contains benzyl derivatives that favour the production of penicillin II in preference to penicillin I. Since the adoption of corn steep liquor for routine production in this country, penicillin II is also the predominant form in British penicillin. Another form of penicillin produced by some strains of *P. notatum* and *P. chrysogenum*, especially in presence of the appropriate precursor, is penicillin III or penicillin X, in which R is a *p*-hydroxy benzyl group. Even under the best conditions, however, it is produced in limited amounts only, but, as it has rather unusual biological properties, interest in this form of penicillin may be expected to increase.

In 1945 a new strain of *P. chrysogenum*, known as Q.176, was generally adopted for submerged culture, because it produced much larger quantities of total penicillin than the strain X.1612 previously employed. It was not immediately realised, however, that, in absence of precursors, the predominant form of penicillin so produced is not the therapeutically valuable penicillin I or penicillin II, but another penicillin termed penicillin K, in which R is a *n*-heptyl group. This form of penicillin is rapidly inactivated in the animal or human organism and is therefore clinically less effective than penicillin I or II. Even in presence of precursors, substantial amounts of penicillin K are produced. The relative effectiveness of the various forms of pencillin is still a matter of controversy, but the balance of evidence is in favour of reducing to a minimum the proportion of penicillin K in material to be used for clinical purposes.

A substance isomeric with penicillin I and with similar biological properties is produced by *Aspergillus flavus* and has been given the names flavicin and flavicidin.[2] In this substance R is a Δ^3-pentenyl group.

The discovery that different forms of penicillin do not have the same therapeutic value has made it imperative to find methods of estimating the individual penicillins in commercial penicillin, and this is proving to be an extremely difficult task. For a long time the only method of estimating the amount of total penicillins in a mixture was the bacteriological method of assay, and although this has been improved so that the errors of the method now in use are surprisingly small, such a method must always suffer from certain practical disadvantages: for example, that the result can only be known several hours after submission of the sample for test, and that occasional highly erroneous results are obtained. Within recent months several chemical methods of estimating total penicillin have been described. Scudi,[3] for example, published details of a colorimetric method and of a fluorimetric method of assay based on the fact that the β-lactam ring of all the penicillins is opened by organic bases to form substituted amides and, by using an amine containing a chromophoric group (N-(1-naphthyl-4-azobenzene)-ethylene diamine) or an amine containing a fluorophoric group (7-methoxy-2-chloro-5-β-aminoethylamino acridine), products which are either coloured or fluorescent are produced and can be estimated by conventional procedures. These methods have very serious limitations, due mainly to the difficulty of completely removing excess of the reagent from the coloured product; moreover, they are tedious and the reagents are difficult to prepare.

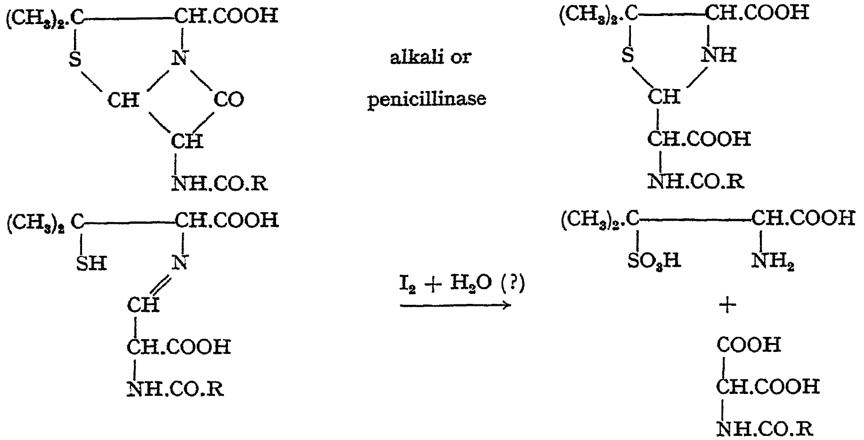
Reactions of this type can be represented as follows:



A similar reaction occurs with hydrazines and hydroxylamines and can likewise be utilised for the quantitative estimation of total penicillins. Such methods, however, cannot be used for the differentiation of individual penicillins, as these all behave in the same manner towards these reagents.

A simpler method of estimating total penicillins is based on the fact that, on treatment with alkali or with a specific enzyme known as penicillinase, the β-lactam ring of penicillin is opened, with the formation of an additional carboxyl group, giving penicilloic acid; the amount of alkali required to neutralise the acidity thus produced is proportional to the penicillin content. These two methods are not very accurate, though the penicillinase modification is, as might be expected, highly specific. An improved procedure, which is likely to prove more useful than either of Scudi's methods, is the iodimetric method of Alicino,[4] which is based on the observation that the difference in the amount of iodine consumed under standard conditions before and after inactivation by alkali is proportional to the amount of penicillin present. The alkali converts penicillin into penicilloic acid, which adds on six to nine equivalents of iodine, depending on the conditions used. The mechanism of the reaction is obscure but, provided the conditions are rigidly standardised, the amount of iodine reacting is always constant. The reaction undoubtedly depends on the fact that the thiazolidine ring in penicilloic acid is readily opened to form a free thiol group, whereas ring-cleavage does not occur with compounds, such as penicillin, in which the thiazolidine nitrogen atom is acylated. The free thiol group is then oxidised by the iodine, presumably to a sulphonic acid group, whilst an aldehyde group is probably liberated and then oxidised as in the scheme on p. 276.

The bacteriological method of assay, in addition to its purely practical disadvantages, also suffers from a very serious theoretical objection, for not only do the individual penicillins differ in therapeutic value, but they also differ in the extent to which they inhibit the growth of test-organisms. The degree of inhibition produced by a mixture of penicillins is therefore dependent on the proportions in which the constituents occur, and the biological assay may give an entirely different result from the chemical method of assay and probably neither will give a satisfactory estimate of the therapeutic efficacy. The proportion of two penicillins in a mixture can be determined from the ratio of the response of two different organisms, such as *Staphylococcus aureus* and *Bacillus subtilis*, but the method is not very accurate and is invalid if a third penicillin is present; with commercial preparations, this is at present the rule rather than the exception.



It is important, therefore, that satisfactory methods of estimating the individual penicillins should be made available as soon as possible. The only chemical method that has so far given satisfactory results is that of Sheehan *et al.*[5] for the estimation of penicillin II. This method depends on the observation that penicillin II forms with N-ethyl-piperidine a sparingly soluble salt which can be filtered off and weighed. The results are said to be quantitative with material containing not less than 50 per cent. of penicillin II and with a potency not less than 800 units per mg., but in our experience the results may not be reliable with material containing less than 90 per cent. of penicillin II. The method has recently been improved in our laboratories by combining the method of Sheehan *et al.* with that of Alicino. A colorimetric method of estimating penicillin II, actually a modification of the Kapeller-Adler method for the estimation of tyrosine, was described by Page and Robinson[6] but is less specific than the method of Sheehan *et al.*, since impurities containing aromatic rings also react, giving rise to coloured products and thus leading to high results. The same disadvantage applies to spectrophotometric methods of estimating penicillin II that depend on the absorption of the phenyl ring, although a method recently elaborated by Philpotts, Thain, and Twigg[7] has given valuable results with material of high potency.

The only chemical method available for the estimation of penicillin K is one recommended by the Food and Drug Administration of the U.S.A., but it does not give reliable results. In this method the penicillin is distributed between a buffer solution of *p*H 6 and chloroform; most of the penicillin K goes into the chloroform phase, whilst most of the penicillin G remains in the aqueous phase. The amount of penicillin in the chloroform solution is estimated by iodimetric titration. Unfortunately the separation is not sharp and other penicillins present may be distributed more equally between the two phases and so increase the error of the method.

The method to be described by Dr. Goodall and Dr. Levi is, like the foregoing, a distribution method, but is of far greater value, since all the penicillins in a mixture can be estimated simultaneously. Morevoer, it is capable of indicating what penicillins are present, so that a qualitative and quantitative analysis of a mixture may be made at the same time. It is without doubt the most useful method yet described for the examination of mixtures of penicillins.

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A Micro-chromatographic Method for the Detection and Approximate Determination of the Different Penicillins in a Mixture

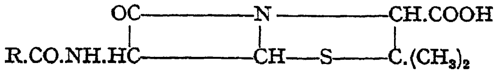
BY R. R. GOODALL AND A. A. LEVI

(Read at the meeting of the Society on May 7th, 1947)

INTRODUCTION—The following Table contains data (as available in February, 1947) on certain characteristics of the penicillins.

TABLE I

PENICILLINS: NOMENCLATURE AND TYPE OF SIDE-CHAINS



$$\begin{array}{llll} & \text{OC}\text{------} & \text{N}\text{------} & \text{CH.COOH} \\ & | & | & | \\ \text{R.CO.NH.H} & \text{C}\text{------} & \text{CH---S---} & \text{C.(CH}_3)_2 \end{array}$$

Name			Activity per mg.		
British	American	Side chain (R)	*a*, against *B. subtilis*	*b*, against *S. aureus*	Ratio $a \div b$
I	F	Δ^2 pentenyl	970	1550	0·63
II	G	benzyl	1667	1667	1·0
III	X	*p*-hydroxy benzyl	800	900	0·89
K	K	*n*-heptyl	700	2300	0·30
IV	—	unknown	unknown	unknown	—
VI	—	”	”	”	—
VII	—	”	”	”	—

NOTES—

(a) Penicillin IV is not identical with penicillin K as stated by several authors.[7]

(b) Penicillin K is probably identical with a penicillin originally called V (five) by ourselves, but this cannot now be confirmed.

(c) The activities given are those currently available.[8] Differences due to strain variation may be observed in practice. Values for penicillin K are based on unpublished data.

(d) There is some uncertainty about the *B. subtilis* activity of penicillin III (*cf.*[8]).

Commercial samples of penicillin may contain from at least three to seven individual types, the proportions of which may vary widely, depending on the strain of organism, method of culture and method of isolation. Although the penicillins hitherto isolated in a pure state (Table I, I to K) are not widely different in chemical structure, their therapeutic values are not identical, partly because of differences in chemical stability and partly because of possible differences in biological activity against different test organisms.

Methods for determining certain of the penicillins in admixture with others have already been published. Schmidt, Ward and Coghill[8] show how the composition of a two-component mixture can be deduced from the *B. subtilis/S. aureus* assay ratio. The differential assay principle has recently been extended by Higuchi and Peterson[11] to mixtures of three components. Fischbach, Mundell and Elbe[6] describe a macro-chromatographic method for determining penicillin K. Sheehan, Mader and Cram[9] base a method for penicillin II on selective precipitation as the N-ethylpiperidine salt. Penicillin III and K contents can be determined by distribution between appropriate buffer solutions and chloroform.[10]

It is, however, desirable that an analytical method be available for determining directly the proportions of all the different penicillins in a given sample, and this is the aim of the present investigation. We have stated in a preliminary communication[1] that the modified partition chromatogram[2] obtained by using buffer salts in the stationary phase can be adapted to the micro scale, after the manner of Martin and co-workers,[3] who separated a mixture of amino acids and located the position of each component on the paper strip by colour reaction with ninhydrin. In our preliminary attempts to separate penicillin from impurities by the following method, we found that colour reactions were not readily applicable to the treated paper strip, and we therefore tried a biological technique similar in principle to the routine "cup assay" for penicillin. This technique, which is very simple and highly sensitive, had potential quantitative applications, and it was found possible to establish a method for the approximate determination of the relative proportions of the various penicillins present in a given mixture. This method is described below.

METHOD

SPECIAL REAGENTS REQUIRED—

1. *Stock potassium phosphate buffer solution*—Dilute 500 ml. of phosphoric acid of sp.gr. 1·75 with 1500 ml. of water and add potassium hydroxide solution (containing 906 g. in 3 litres of solution) until 2 ml. of the mixture, diluted to 60 ml. with distilled water at room temperature, has *p*H 6·9 ± 0·04 as measured by glass electrode, using, as standard, potassium hydrogen phthalate solution, *p*H 3·97, at room temperature.

2. *Ether*—Shake vigorously 2 litres of ether (anaesthetic quality) with 250 ml. of water for about 2 minutes. Remove the aqueous layer and store the wet ether in the cold room (0° to 5° C.).

3. *Nutrient agar*—Prepare twenty bottles of 285 ml. of filtered sterile medium containing peptone 10 g., Lemco 3 g., sodium chloride (A.R) 2 g., and agar 25 g. per litre of aqueous solution. Adjust to *p*H 7·5 with 2 *N* sodium hydroxide before filtration.

4. *Buffered filter paper*—Wet Whatman Filter Paper No. 4 with 30 per cent. buffer solution of *p*H 6·9 (Reagent 1). Remove the excess of buffer solution by pressing between sheets of clean blotting paper sandwiched between plate glass. Hang the damp treated paper vertically to dry in the air; it dries in about 2 hours. Cut the dry prepared paper into strips 1·8 by 30 cm. and mark each strip with a pencilled cross on the centre line 6 cm. from one end. The width should be as close to the above figure (1·8 cm.) as possible, and strips with ragged edges should be rejected.

5. *Photographic material*—Extra contrasty gaslight paper (16 by 16 inches), etc.

SPECIAL APPARATUS REQUIRED—

1. *Micro-pipettes*—A. *Automatic pipettes for aqueous solutions*—Draw out thick-walled capillary tubing approximately to the dimensions given in Fig. 1. Cut the glass at point O so as to form two jets. Coat the inside of each jet with paraffin wax. Holding each tube vertically, dip the tip into toluene or xylene. The solvent will rise to a definite height. Remove the toluene by pressing the tip of the pipette on to filter paper. Repeat the toluene treatment four times and then dry the pipette by blowing air through it gently. Dip the tip of the pipette into distilled water and note that the liquid rises by capillarity to the boundary of the paraffin coating. Discharge the pipette by pressing gently on to filter paper. Cut down the jet of the pipette until the amount of water delivered in this way is constant at from 1 to 1·5 mg., and mark the position of the liquid meniscus.

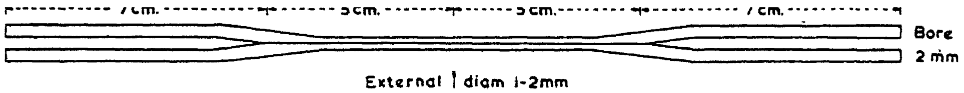


Fig. 1. Dimensions of tubing for automatic micro-pipettes, type A.

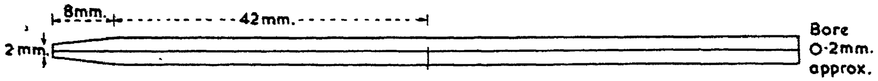


Fig 2. Approximate dimensions of capillary micro-pipette, type B.

B. *Capillary micro-pipettes for solvent extracts*—Prepare a 1-microlitre glass pipette as shown in Fig. 2.

2. *Apparatus for chromatographic development*—The apparatus is illustrated in Figs. 3A, 3B, and 3C.

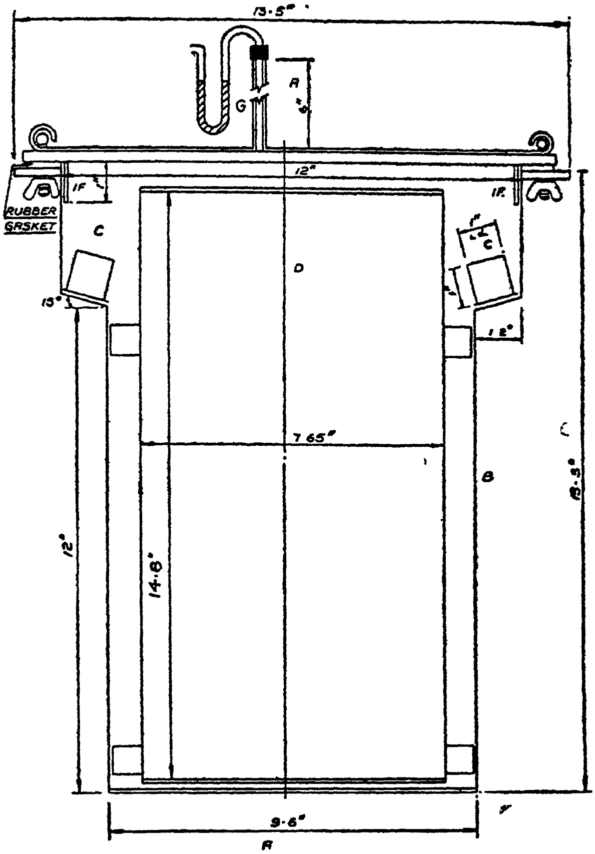


Fig. 3A. Section through 'A' 'A' of apparatus for development of 24 paper chromatograms.

B. Chamber with lid and internal flange, IF., secured by 8 wing nuts on to a soft rubber gasket.

C.C. Ring carrying 24 cups seating on a shoulder 3" from top of chamber.

G. Stainless steel vent tube ($\frac{1}{4}$" O.D.) attached to a short U trap containing mercury.

D. Closed drum with 4 spacing pieces.

Material of construction — body-welded stainless steel sheet 20 gauge, flange and lid $\frac{1}{4}$" stainless steel plate.

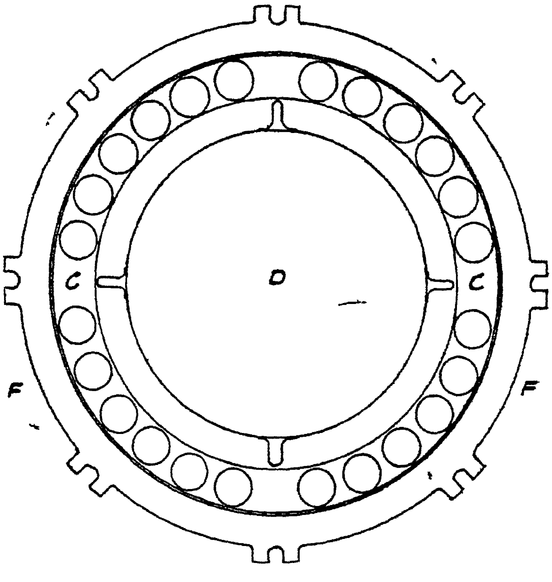


Fig. 3B. Plan of top of chamber with lid removed.

F. Flange with 8 lugs for wing nuts.

C.C. Ring, showing distance between centres of the cups.

For true position of each cup, which is inclined inwards (at an angle of 15°), see Fig. 3A. Each cup holds a glass reservoir (see Fig. 3c).

The vertical surface of the closed drum D (Fig. 3A) is tightly wound with one thickness of cotton bandage, 2 inches wide, and each end is stitched to the adjacent lap to prevent unwinding. Lengths of rustless spring curtain rod (about 23·5 inches long before expansion) are then clipped over the bandage, at 2-inch intervals, to form a set of eight horizontal rings. The bandage is moistened before the apparatus is to be used; the spring rings prevent the paper chromatographic strips from coming in contact with the wet cotton.

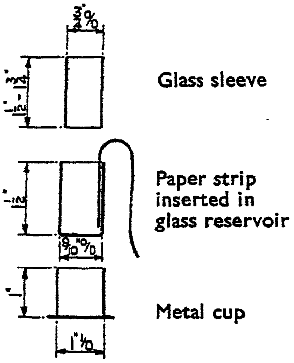


Fig. 3c. Details of glass reservoir, etc., before assembly.

3. *Glass plates for agar medium*—Construct these as detailed in Fig. 4 by cementing plate glass strips, 0·75 inch wide, to a square plate-glass base, 16 by 16 inches, with shellac dissolved in alcohol. Each plate will carry seven chromatographic strips, or six and a strip with standards. Provide a plate-glass lid, 16 by 16 inches, for each of the plates.

A sheet of plate glass, 13·5 by 13·5 inches, just smaller than the space for the agar in the above plates, will also be required.

Clean and sterilise each plate before use (see below).

4. *Dilution pipette*—In general, sufficient sample will be available to permit serial dilution by graduated pipettes. If, however, economy of sample is a prime consideration, the technique of the dilution pipette[5] is readily applicable.

These pipettes must not be used for organic solvent solutions.

5. *Burettes for charging ether*—Prepare two rough burettes holding 60 ml. and graduated at intervals of 8 ml. Control delivery of liquid by a spring clip on a rubber tube of 5 mm. bore.

6. *Vessel for humidifying strips*—Obtain a large vessel (*e.g.*, a large drum) with a lid. Provide means of keeping the atmosphere in this vessel damp (*e.g.*, a damp cloth hanging round the inner circumference). Provide a stand on which the strips can be hung, well spaced out from each other, inside the vessel, so as to expose them freely to the atmosphere of the vessel.

PROCEDURE—

FIRST DAY

Preparation of sample and standards—Prepare a solution of the sample containing from 1 to 40 units per microlitre. Dissolve the sample in half the calculated volume of water and make up to volume with 30 per cent. buffer solution of *p*H 6·9 (Reagent 1). Filter or centrifuge if necessary; 50 to 100 μl. of solution are ample.

For standards make five serial dilutions of a solution of pure sodium penicillin II containing approximately 30 units per ml. in 30 per cent. *p*H 6·9 buffer solution (Reagent 1), each one-third the strength of the previous solution, one volume of solution being diluted with two volumes of buffer at each stage.

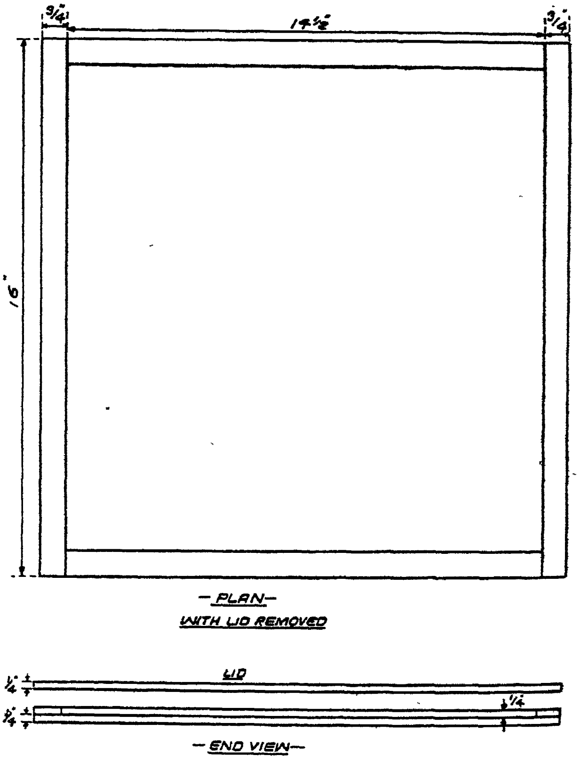


Fig. 4. Dimensions of plate glass for agar medium.

All solutions and standards must be kept stoppered in the cold room (0° to 5° C.) except when actually required for use. After 14 days fresh solutions should be made up.

Solvent extracts and samples already in solution—These are used without further treatment. Waxed pipettes must not be used for organic solvent extracts.

"Spotting" solutions of sample, etc.—Fifteen minutes before beginning "spotting," place the required number of strips in the special apparatus 6 to humidify. After this treatment, apply to each strip a 1-μl. spot of the test solution at the marked point. Fix a wire paper-fastener to the bottom end of each strip (*i.e.*, the end remote from the marked point). Remove the central drum from the special apparatus 2. The apparatus should be at room temperature during this operation. Place the top end of each spotted test strip in a glass reservoir supported in the frame of the apparatus and fix it in position by inserting the glass sleeve provided for each reservoir. Ensure that the top end of the strip just touches the bottom of the reservoir and that the "spot" is located about 1 cm. below the edge of the reservoir. Adjust the strips so that the clipped ends hang vertically, *i.e.*, clear of each other and not touching any part of the apparatus.

Put 50 ml. of ether in the bottom of the apparatus to form a pool. Remove the apparatus to the cold room. Wet the cloth apron round the central drum with water and carefully place the drum in the apparatus. Replace the lid.

After 2 hours, and while the apparatus is still in the cold room, remove the lid. Deliver 8 ml. of ice-cold ether into each reservoir. Without delay, replace the lid on the apparatus and make gas-tight. This operation must be carried out as quickly as possible to avoid formation of ice on the strips.

Allow development to proceed overnight.

Cleaning and sterilisation of glass plates—After removing used agar, rinse the plates thoroughly with water. Wipe the surface with dilute hydrochloric acid (0·4 per cent.). Allow the acid to remain on the plates for 2 to 3 minutes, rinse with water, wash with soap and water, and again rinse with water. Careful cleaning of the plates in this way is essential.

Support the plates on top of an asbestos sheet, using cork spacers, and sterilise by heating at 120° to 130° C. for 3 hours. Store the required plates at 37° C.

Spotting out standard solution—Prepare one standard strip carrying undeveloped spots by delivering 1-μl. spots of each solution on to a *p*H 6·9 buffered strip at points 5 cm. apart along the strip. Store in the cold room until required.

SECOND DAY

Melt the agar medium and inoculate it with *B. subtilis* spores at a temperature not below 70° C.; shake and allow the froth to break at 70° C. Pour the medium on to an uncovered glass plate (special apparatus 3) just removed from storage at 37° C. and laid on a level glass surface. If necessary, flame the plate locally to assist covering by medium. Protect the medium with the plate lid, spaced 0·5 inch above the surface: allow to set and evolve moisture during 10 minutes. Then lower the lid on to two felt strips, placed on opposite sides of the plate, invert the plate and allow to cool during 15 minutes in an asbestos-lined box in a cold room (0° to 5° C.).

Plating out the strips—Open the apparatus containing the developed chromatographic strips, remove the central drum carefully and transfer the strips to a clean glass container. If any reservoirs still contain ether, remove the strips from these first, otherwise ice will collect at the top of the strips owing to evaporation of ether.

When the agar plates have been in the cold room for at least 15 minutes, begin plating out the strips in the following way.

Support the 13·5 by 13·5 inch sheet of plate glass (special apparatus 3) on blocks so that it is raised off the bench about 2 inches and the edges are not obstructed in any way. Clean the surface with acetone. Arrange a set of strips evenly on the top of the glass. A card ruled at 1·9-inch intervals from the centre and attached to the under-side of the glass is a convenient guide. Lift the agar plate off the felt supports and lay it gently, face down, on top of the strips. When the strips have wetted out (this is easily observed) lift up the agar plate. The strips will adhere to the agar surface in their correct positions. Replace the agar plate on the felt supports on the lid and transfer with its lid, still with the agar face downwards, to the cold room.

Incubation—After the plates carrying the strips have been in the box in the cold room for four hours, close the plates with the lids by removing the felt strips and place, face downwards, in an incubator at 37° C. Allow incubation to proceed overnight.

THIRD DAY

Reproducing the results—Remove the plates from the incubator and identify the strips with "Chinagraph" markings on the glass. Using the patterns on the plates as a negative, make contact prints on the gas-light paper.

Measurement of zones—Measure the diameters of the circles given by the stationary (undeveloped) standards, estimating to 0·1 mm. Similarly measure the maximum width of each zone on each developed strip.

CALCULATION OF RESULTS—

Relative proportions of B. subtilis *units*—Let the diameters of the circles given by the standard spots be A, B, C, D, E, and F in descending order of magnitude. Then the slope b' (compare conclusions following Table II, p. 286) for the developed zones is given by the expression:

$$b' = 1{\cdot}2\,[5(A-F) + 3(B-E) + (C-D)]/35 \log 3$$
$$= 0{\cdot}0719\,[5(A-F) + 3(B-E) + (C-D)]$$

Divide the diameter (in mm.) of each developed zone by b'. The resulting value is the logarithm of the number of arbitrary units in the zone. Calculate the mean activity in arbitrary units for each zone, and from these obtain the percentage composition. The arbitrary *B. subtilis* units apply to the particular plate only.

Conversion to relative proportions of S. aureus *units*—

(i) Divide the per cent. activity in each zone (*B. subtilis* units) by the appropriate factor (Table I).

(ii) $$\frac{\text{Units against } B.\ subtilis}{\text{Units against } S.\ aureus} = \frac{100}{\text{Sum of quotients from (i)}} = F$$

(iii) Proportionate activity (*S. aureus* units) in each zone
$= F \times \%$ activity in *B. subtilis* units.

Composition by weight—

(i) $$\text{Per cent. of total activity in each zone} = \frac{\text{Per cent. activity in } B.\ subtilis \text{ units}}{\text{Activity* } (B.\ subtilis \text{ units}) \text{ of the appropriate pure component.}} = P$$

(ii) Per cent. of total sample weight in each zone
$= P \times$ activity of the sample in *B. subtilis* units.

Note—For penicillins of undetermined activity the ratios *B. subtilis/S aureus* have been assumed to be unity, and the activity 1000 units per mg. Where these penicillins occur only as minor components of a mixture, as they have done in many samples we have examined, the error introduced into the over-all analysis by this approximation is probably not great.

RESULTS AND DISCUSSION

As a necessary preliminary, it was established, from the results of a series of experiments designed to detect a loss of 7 per cent. or more of the total activity, that no significant loss took place when the above method was applied to a blend of calcium penicillin (activity 300 units per mg.). Losses, however, occurred if the chromatograms were carried out at room temperature or if serious delay arose before completing the experiment. In these experiments the active zones were stripped from the paper with water and the activity of the resulting solution was compared with that of a freshly prepared solution of the same sample.

QUALITATIVE SECTION

The chromatographic identification of the zones (Fig. 5, A) obtained from an early sample, by reference to the results obtained from known penicillins developed simultaneously on adjacent strips, is illustrated in Fig. 5, A, B and C. The zone A II is attributed to penicillin II not only because it corresponds in situation to the zone C II obtained from pure penicillin II, but also because the increment in zone diameter (B II–A II) due to enrichment of the sample with pure penicillin II is confined to the one appropriate zone and is of the expected order of magnitude, according to later deductions. Since the other major constituent of this sample was known to be penicillin I, by the macro-column technique, the identity of the substance producing the other zone in Fig. 5, A is in all probability penicillin I. The identity of penicillin III on the paper strip was established by similar comparative tests using a penicillin III fraction from a macro column. Resolution of penicillins II and III is shown in Fig. 5, D. The above three penicillins all occur in the same order as observed on macro columns.

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* See Table I.

When the technique came to be applied to products from various sources, further zones of activity were resolved (*e.g.*, Fig. 5, E). Ideally, for the qualitative and quantitative development of the technique, the behaviour of mixtures of the pure individual penicillins should be studied, but only penicillin II was available in the pure state. However, there was little difficulty in identifying the various zones of activity, from the fixed order in which they occur after development. The behaviour is analogous to that of the amino acids reported by Martin and others.[3] Penicillin II was a convenient key zone, for it was usually known to be the major active constituent; if not, it was added as a "tracer," in the manner of Fig. 5, B. The order from the point of application of the solution is: III, VI, II, I, IV, VII and K. The seven zones were detected on one strip (*cf.* reproduction in Fig. 5, E). The existence in Fig. 5, E and Fig. 6, C of the zone attributed to penicillin VII has been repeatedly confirmed. It is also apparent in Fig. 6, C that there is a suggestion of further resolution at the front of the K zone. The existence of penicillin VI was clearly demonstrated in other experiments as shown in Fig. 6, A and B. Penicillins VI and VII have only been found in minor amounts, generally less than 5 per cent. of the activity of unfractionated material.

Work on macro columns in these laboratories (to be published shortly) has confirmed that the zone usually attributed to penicillin I is due to a mixture of two penicillins, called I and IV (penicillin IV is not identical with penicillin K). This is also clearly shown by the present technique (see Fig. 5, E).

It is known that penicillin K is the fastest-moving penicillin.[6]

With further experience it has been found that the method of identification based on ratio of distances traversed by unknown and known "spots"[1] is less satisfactory than was at first thought. Following are comments on certain aspects of the technique.

CONTROL OF DEVELOPMENT

(1) *Humidity*—It cannot be stressed too strongly that the humidification treatment of the strip before and after spotting is of cardinal importance. For example, a prior treatment of the strip for one hour instead of 15 minutes will result in elongation of each zone, the K zone partly overshooting the end of the strip. The syrup obtained by evaporation of buffer solutions of the type used is very sensitive to the state of humidity of the atmosphere. The best method of controlling the moisture content of the strip was found to be to expose the treated paper to a fully humid atmosphere for a specified time. Even this is not entirely satisfactory.

(2) *Concentration of buffer*—This also has very marked effects on distance of development. Strips were impregnated with phosphate buffer solutions ranging from 1 per cent. w/v up to 30 per cent. w/v (Reagent 1) and it was found that the use of the 30 per cent. w/v solution, which is about the maximum convenient concentration, led to zones of the most compact and most regular type; it also produced the farthest separation of the zones, in other words the greatest degree of resolution, obtained.

(3) *pH value*—For the slower-moving zones, variations of ± 0·1 unit in the *p*H of the applied buffer solution have little effect, but a closer control of *p*H is desirable in order to retain the fast-moving zones on the strip. The method outlined above lays down conditions adequate for separating the major constituents, I, II, III and K. For the detection of traces of other penicillins (*e.g.*, VI) some variation in the conditions may be necessary. For example,[1] the existence of the new penicillin VI was revealed between penicillins III and II when a chromatogram on a strip carrying buffer of *p*H 6·78 was repeated on a strip carrying buffer of *p*H 6·25 (*cf.* Fig. 6, A and B).

(4) *Width of strip*—The width specified is a compromise between narrow strips (10 mm. wide) which are more productive of faults such as asymmetric zones (Fig. 5, D II), and wide strips (20 mm. wide), which obscure the appearance of the smaller zones and are an inconvenient size for fitting into the apparatus. Strips must be of uniform width and free from ragged edges. The latter are liable to cause gross irregularities. A mean change in strip width of 1 mm. caused a mean change in zone diameter of the order of 0·4 mm.

(5) *Solvent*—Of the available solvents, ether has been by far the most satisfactory. Adjustments of *p*H will be necessary if other solvents are used.

(6) *Development*—The apparatus (Figs. 3A and 3B) is designed to standardise as far as possible the degree of development, which is related to throughput of solvent and moisture content of the treated strip. In order to keep these two factors constant, the apparatus is made gas-tight, with individual reservoirs for each strip and with a large damp surface to keep the atmosphere humid. The junction at the flange and lid is readily made gas-tight by means of a Neoprene sponge rubber gasket.

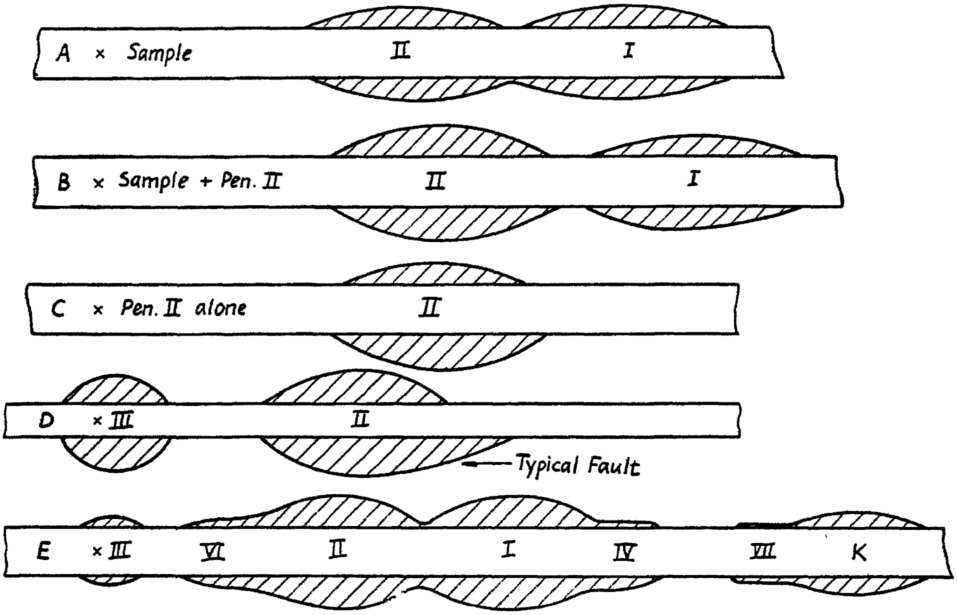

Fig. 5. × = Point at which penicillin solution was applied. The area where growth is inhibited is shaded.

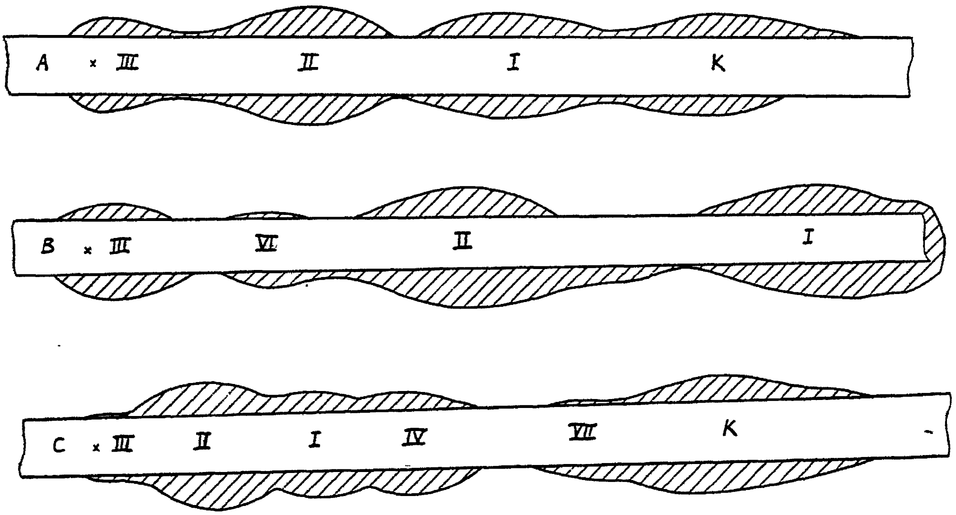

Fig. 6.

BIOLOGICAL PROCEDURE

(1) *Precautions regarding sterility*—These were confined to the preparation and use of the biological plates, since no practical advantage was gained by sterilising the paper strips before use. Little infection was encountered under the specified conditions.

(2) *Medium*—Inoculated agar sheets of uneven thickness are useless for quantitative work, owing to marked variation of zone diameter with depth of agar. Hence the molten medium must be poured under conditions that permit the agar to form a plane sheet before setting.

(3) *Diffusion*—Larger zones with clearer edges are obtained if a period is allowed for diffusion before incubation. This is not, however, essential. Care must be taken that there is no temperature gradient across the plate. For example, from a plate containing only undeveloped zones kept cold in a domestic-type refrigerator, there was a significant variation in zone diameter across the plate from the side nearest to the cooling coils.

QUANTITATIVE

For the development of a quantitative technique, dimensions such as area or length of the inhibition zone were too highly dependent on degree of development and also were not precise when the zones overlapped. However, these objections did not apply to the maximum diameter of the zone, which in preliminary experiments appeared to be proportional to the logarithm of the units in the zone. The precise relationship defining the diameters of the inhibition zones obtainable both from developed and undeveloped spots was investigated in the following way.

A set of solutions of penicillin II crystalline sodium salt (Table II) was prepared by serial dilution. Using 1-μl. spots from each of these solutions a set of strips carrying 1-μl. developed spots were obtained after chromatographic development. These developed spots were arranged on a biological plate together with a set of 1-μl. undeveloped spots from the same set of solutions. The diameters of the resulting zones of inhibition are given in Table II. The experiment was carried out under routine conditions without special supervision, and the figures are probably fairly representative.

TABLE II

COMPARISON OF INHIBITION ZONE DIAMETERS (MM.) OBTAINED FROM "UNDEVELOPED" (U) AND "DEVELOPED" (D) SPOTS OF KNOWN ACTIVITY

	Sodium penicillin II solution; units/ml. (*B. subtilis*):								
	10·47	3·49	1·163	0·388	0·129	0·043	Mean (*a* and *a′*)	Slope (*b* and *b′*)	*b′*/*b*
U	44·4	39·6	37·1	32·1	29·9	23·9	34·50	8·18	1·20
D	43·2	41·0	35·8	30·2	26·9	20·0	32·85	9·82	
U	43·2	40·2	36·9	33·2	27·8	22·2	33·92	8·75	1·08
D	43·2	40·6	36·4	30·2	25·1	22·2	32·95	9·41	
U	43·6	39·7	37·2	32·6	29·6	24·2	34·48	7·91	1·24
D	43·2	41·2	34·8	31·1	27·9	19·2	32·90	9·80	
U	42·9	41·4	37·3	33·6	28·5	24·8	34·75	7·96	1·19
D	44·0	40·5	36·8	31·6	27·2	21·5	33·60	9·44	
U	44·9	40·8	38·1	33·0	31·0	24·8	35·43	7·66	1·20
D	45·4	42·0	36·2	32·5	28·2	22·1	34·40	9·77	
U	46·5	43·0	39·8	35·1	31·1	25·0	36·75	8·86	1·10
D	44·0	43·8	37·8	33·1	30·0	20·8	34·93	9·69	
U	43·9	40·7	37·2	32·8	29·3	24·8	34·78	7·66	1·16
D	45·1	41·1	36·5	31·9	26·4	23·7	34·11	8·86	
U	38·3	36·7	33·1	26·8	26·1	21·7	30·45	7·25	1·22
D	40·0	36·9	32·3	28·5	23·8	19·1	30·11	8·87	
U	39·1	36·5	33·0	28·8	25·2	21·2	30·63	7·64	1·16
D	39·5	36·7	32·7	29·1	23·0	18·9	29·99	8·86	

Notes on Table II—

a and *a′* are ordinates, *b* and *b′* are slopes for six results for undeveloped and developed spots respectively. Standard deviation of each zone diameter: U, 0·9; D, 1·24 mm.

The following conclusions were drawn from the data:

(1) For both sets of spots the trend of the results is largely accounted for by an expression of the type

$$\text{diameter of zone} = a + b \log (\text{units in zone})$$

where a and b are constants for a particular biological plate.

Both a and b may differ considerably between plates.

(2) There is, however, a small but significant curvature, which is similar in magnitude for both sets, *i.e.*, the relationship is only approximately rectilinear. A similar result has been observed in the ordinary cup assay when carried out with an extended range of concentrations.

(3) The slopes for the undeveloped zones (b) were significantly lower than those for the developed zones (b').

(4) The ratio of the slopes b'/b was sensibly constant, with a value of approximately 1·2; similar values were obtained on several other occasions.

(5) The over-all standard error, including that due to the curvature, is of the order of ± 1 mm. This corresponds approximately, at 95 per cent. limits of error, to ± 25 to 30 per cent. in the estimation of activity in any one zone. For a sample replicated on 6 strips the 95 per cent. limits of error are thus about ± 15 per cent.

In a further experiment in which solutions containing 30, 3, and 0·1 units were each developed with 1, 3, 5, 7, 9, and 11 ml. of ether, it was shown that zone diameters increased with distance of development for the strongest solution, while the reverse was true for the weakest solution. No effect was detectable at the intermediate strength. This phenomenon accounts for observation (3) above.

OTHER POSSIBLE METHODS OF DETERMINING PLATE SLOPE—

The method given above was adopted after considering three alternatives.

(*a*) Comparison with the results from standard mixtures of the pure penicillins so arranged as to give series of developed inhibition areas appropriate to each penicillin. This is not possible in the absence of the pure components.

(*b*) A planned distribution of the developed test spots and the undeveloped standard spots over several plates. This method is dependent on reasonable conformity of each plate to an average plate slope, but plate to plate variations of slope, even on a single day, were too great.

(*c*) Serial dilution of the sample itself. As the minor zones soon disappeared in the results obtained from a set of serial dilutions, and as there is obviously an upper limit to the load (governed by the area available on the biological plate), this procedure did not permit adequate replication. Nevertheless if samples of suitable composition are available this method should provide useful information about the relative plate slopes of the different penicillins.

In adopting the undeveloped spots as standards, the advantage that the results are, comparatively, of a high order of accuracy will counteract the errors due to the uncertainty of the empirical factor 1·2 used to calculate the slope for the developed spots. Small errors in the value of the slope are important only in connection with very small or very large zones, as, for example, in calculating the proportions of an impurity in a major component.

It is probable that the slopes of the developed zones increase from pencillin III to penicillin K, but the evidence so far obtained is not conclusive. Since penicillin II is developed a moderate distance down the strip, the value of b' for penicillin II is probably a reasonable value for the mean of slopes. Errors due to the use of this figure can be expected; thus differences between proportions of penicillin III in different samples will be minimised; differences between proportions of penicillin K will be exaggerated.

TYPICAL NUMERICAL RESULTS—

In the absence of samples of the pure components for making synthetic mixtures, checks on accuracy have had to be confined to such results as are shown below in Tables III, IV and V.

TABLE III

REPLICATION OF ANALYSES BY ROUTINE OPERATOR

	Pencillins found:						
	III	II	I	IV	VII	K	
Sample 1:							
Six-strip assay	0·5	37·5	29·8	19·7	1·4	11·1	per cent.
Repeat	1·1	39·6	28·5	17·7	trace	13·1	„ „
Sample 2:							
Six-strip assay	1·9	15·6	18·8	16·4	—	47·0	per cent.
Repeat	1·8	15·9	22·5	17·8	—	41·7	„ „

TABLE IV

COMPARISON OF CALCULATED AND OBSERVED VALUES FOR DIFFERENTIAL ASSAYS

The figures below represent values of the ratio: units against *B. subtilis* ÷ units against *S. aureus*, for a variety of samples containing from 3 to 7 component penicillins.

Observed:	0·65	0·71	0·98	0·82	0·58	
Calc. (see method):	0·69	0·71	0·98	0·88	0·55	
Observed:	0·83	0·94	0·85	0·80	0·89	0·68
Calc.:	0·89	0·96	0·89	0·86	0·92	0·75

TABLE V

MATERIAL BALANCE (MEGA-UNITS) ON PARTIAL FRACTIONATION OF A PENICILLIN SAMPLE

Fraction No.	Total mega-units (direct assay)	Composition of each fraction, in mega-units calc. from micro-chromatographic analysis and direct assay III	II	I + IV	K
1	0·079	0·079*	—	—	—
2	0·218	0·002	0·175	0·038	0·003
3	0·507	—	0·446	0·060	0·002
4	0·071	—	0·008	0·062	0·001
5	0·532	—	—	0·523	0·009
6	0·212	—	—	0·160	0·052
7	0·477	—	—	0·015	0·462
Total	2·096	0·081	0·629	0·858	0·529
Starting material	2·40	0·068	0·652	1·17	0·503

* Assumed to be penicillin III, not analysed.

Note—The recovery in a fractionation process of this kind is expected to be about 80 per cent.

The data in Tables III to V are fairly representative of the results obtainable under routine conditions. The method described has proved to be very useful, particularly for the examination of complex mixtures of the type shown in Table III, which, for the first time, can now be directly analysed for every component. It is hardly likely that such a complete analytical picture could be obtained by other than chromatographic means. Bearing in mind that the procedure involves both a chromatographic stage and a biological assay, the reproducibility obtained is reasonably good.

The application of the method to samples predominantly penicillin II has recently become of special interest, since clinical-quality salts containing from 90 to 100 per cent. of penicillin II are now being manufactured. In this connection it is pertinent to illustrate how the figure for the relative proportion of penicillin II is not greatly affected by comparatively large errors in the units found in each of the several penicillin zones. For example, let us assume that a sample of relatively pure penicillin II has the following composition in *B. subtilis* units per cent.:

Penicillins: III, 1%; II, 94%; I, 2%; IV, 1% and K, 2%.

Let us further assume that the error in the estimation of the penicillin II zone be ± 15 per cent. (see p. 286), but that the errors in the minor (III, I, IV and K) zones be *as much as* ± 50 per cent. Now although these deviations are large when considered as absolute errors on each individual unitage, it can be calculated that for mixtures of the above type, the *extreme range* for the *relative* proportions of the major constituent (pencillin II) carries a much smaller error. In the case assumed the range is from 90·0 to 97·3 per cent., where the true figure is 94 per cent.; *i.e.*, the *extreme* errors in the relative proportion are − 4·0 and + 3·3 per cent. It is shown below that the reproducibility in the proportions of penicillin II found in replicate analyses (6-strip assays) of this type is well within these limits:

Pencillins present	III	II	I	IV	K	
Sample 1:	trace	98·2	1·0	0·5	0·4	per cent.
Repeat:	0·4	98·0	0·7	0·3	0·6	„ „
Sample 2:	0·7	93·0	3·3	0·9	2·0	„ „
Repeat:	0·6	94·5	2·5	1·1	1·3	„ „

Hence the method serves to provide a reasonably accurate measure of the relative content of the major penicillin in this class of sample.

SUMMARY

A method is described in which the various penicillins in a mixture are separated by a micro partition chromatographic technique, based on differences in distribution of the penicillins between ether (mobile phase) and phosphate buffer (stationary phase) supported on a strip of filter paper. The separated penicillins are invisible, but after incubation in contact with an agar sheet, pre-inoculated with *B. subtilis* spore suspension, elliptical zones free from bacterial growth are clearly visible in the neighbourhood of each active component. The technique is highly sensitive, 1 μl. of solution (1 to 30 international units) being adequate for a qualitative separation. The maximum diameter of each zone is proportional to the logarithm of the number of units in the zone. Quantitative data are given, indicating the order of reproducibility of the method.

It is shown that the activity in crude products is mainly due to penicillins I, II, III, IV, and K. Small amounts of new penicillins (called VI and VII) have also been separated, but in the samples examined these have never represented more than 5 per cent. of the total activity.

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DISCUSSION

Mr. S. G. E. **STEVENS** congratulated the authors on a method which seemed to have great possibilities in evaluating penicillins and asked whether "halo" formation was observed and whether variations in the concentration of a penicillin introduced any undue vertical as well as horizontal diffusion.

Dr. **LEVI** in reply said that the edges of the zones were quite sharp at all concentrations and halo effects had not been observed.

Dr. E. **LESTER SMITH** said the authors were to be congratulated on this extremely elegant application of the chromatographic technique to a difficult and important problem. It seemed probable that chromatography, and in particular partition chromatography, would become of increasing importance to analysts. The speaker had obtained some experience with the procedure on the basis of the authors' earlier brief report, and had rediscovered some of the snags, in particular the vital importance of humidity control. Another difficulty encountered had been a tendency for the zones, even on the standard strips, to merge into one another as though the paper was taking up too much moisture from the agar jelly and so permitting diffusion of penicillin along the strip.

The paper strip chromatograms were remarkably effective in separating individual penicillins and appeared to give sharper separations than buffered silica gel columns. It was suggested that this might be correlated with the observation that penicillin K sodium salt behaves in aqueous solution as a colloidal electrolyte; its solutions froth badly and are capable of emulsifying organic solvents such as chloroform and solubilising certain dyes that are insoluble in water. Penicillin II sodium salt does not behave in this way in dilute aqueous solution but becomes colloidal in presence of strong buffers. The colloidal particles were likely to be adsorbed by the silica and this could account for the bad "tailing" of penicillin zones on these columns.

It was unfortunate that the nomenclature was so confused. The American system using various arbitrary letters was less tidy than the English numeral system, but the latter was rather spoiled because the number "IV" had been allotted to different penicillins. It had to be conceded that I.C.I. workers were the first to claim this number for a new penicillin in a confidential report, whereas others, in ignorance of this report, had assigned the number to penicillin "K"; perhaps we should agree to use "V" for penicillin "K"? A zone on paper strip chromatograms probably corresponding with the I.C.I. penicillin IV was commonly found with submerged-culture penicillin. It seemed likely that this would be found identical with the dihydro-penicillin I recently claimed by American workers as a component of penicillin made by submerged culture.

The Authors, in reply to Dr. Lester Smith, said they had observed the effects of merging of zones, and diffusion along the edges of the strip, to which he had referred. The main cause was irregularity of the strip edges, but there were undoubtedly other factors still unknown. The effects were certainly less frequent with strong buffer. It was mentioned that it was difficult to cut the strips exactly to size.

Buffered columns on silica gel could give excellent separations, but care must be exercised in the correct choice of initial conditions, such as amount of charge relative to amount of adsorbent, initial concentration, and so forth. Dr. Levi hoped to publish shortly a paper on the theory and practice of this type of chromatogram. Previous publication had been delayed by secrecy regulations.

Mr. F. A. **Robinson** said he had gained the impression that Dr. Levi, in studying the effect of various factors, had carried out factorial design experiments only on the chromatographic procedure; he asked whether similar experiments had been carried out to determine whether any of these factors interacted with factors involved in the bacteriological procedure. It was very difficult to obtain an agar film of uniform thickness, and an irregular film might well introduce errors, wrongly attributed to faults in the chromatographic technique.

Dr. **Levi**, in reply, said that such experiments had been carried out and that the method of pouring the plates was now very rigidly controlled; he did not believe that the variations in the thickness of the film at different points on the plate were sufficient to introduce serious errors, provided that the specified routine was strictly followed.

Dr. **Goodall** said it was important to use a refrigerator so designed as to minimise thermal gradients in the cold atmosphere enclosed. It had been noted that, when a domestic-type refrigerator was used, the diameters of a set of "standard" spots decreased in the proximity of the cooling coil.

Dr. H. **Dryerre**, enlarging on a point made by Mr. Stevens, thought that the thickness of the agar film was a factor which must not be neglected. The test was dependent upon the extent of diffusion of the penicillin lateral to the side of the paper strips. It was reasonable to assume that the penicillin diffused also downwards, and any increase in thickness of the agar layer would by diversion leave less penicillin to diffuse laterally.

In reply, Dr. **Levi** said there was no doubt that thickness of the agar film had a profound effect on zone diameter. Conditions, however, had been worked out which give a reasonably uniform film. The most important condition was that the agar should be above 70° C. when inoculated and poured. If the temperature were, say, 65° C., then visibly irregular films were liable to be obtained.

Dr. A. J. P. **Martin** asked whether the use of a salt solution instead of water in the inner cylinder would not give a more uniform humidity in the chromatogram strips.

Dr. **Levi** said that potassium phosphate solutions of the type used, when evaporated, leave syrups that readily take up or evolve moisture according to the state of the atmosphere. There was, of course, no equilibrium vapour pressure. Salt mixtures had a vapour pressure less than saturation for the particular temperature and tended to dry the buffered paper strips. They were abandoned for this and other reasons. The best method so far found was to expose the strips to a fully humid atmosphere for a specified time. This was still unsatisfactory, as it was difficult to control the state of the strips before humidification was started. Thus strips in the centre of a pile might be more or less damp than those near the top, and so on.

Mr. A. L. **Bacharach** asked whether the iodination of penicillin and its hydrolytic product might not be affected by the nature of the side chain. He also enquired whether it was unnecessary, as he gathered, to sterilise the paper strips before attaching them to the agar plates. The problem of producing satisfactory strips of uniform width was surely one that should be referred back to the makers of the filter paper, particularly if the test and its subsequent modifications were, as a result of the excellent work and description by the authors, to be widely used. He also emphasised the importance, even though the error of these tests should be steadily reduced, of so designing them and computing the results that not only the estimate of penicillin activity, but also its error, could be stated.

Mr. **Robinson** said that the iodimetric method gave very satisfactory results, in the sense that these could readily be reproduced by different workers. He agreed with Mr. Bacharach that the individual penicillins might take up different amounts of iodine; this was especially likely to occur with the *p*-hydroxy benzyl group of penicillin III, though he doubted whether the double bond in the side-chain of penicillin I would add on iodine under the conditions employed.

Dr. **Goodall**, in reply said that he had modified the iodimetric method of Alicino by increasing the nett titre and controlling the reaction temperature by thermostat. The reproducibility was thereby increased. He confirmed the experience of other speakers that it was necessary for each analyst to evaluate the iodine absorption of pure penicillin II, as it varied according to conditions adopted.

It was not necessary to sterilise the paper strips before the chromatographic analysis. A manufacturer had not yet been found to produce strips, but further enquiries would be made in view of possible increased demand.

Further details available in the paper when published would indicate that the error in estimating the activity in any zone was dependent on the type and proportion of penicillin referred to. Until results on known "synthetic" mixtures were available, it was estimated that for a mixture of several components present in similar proportions, the 95 per cent. limits of error were about ± 15 per cent. for 6 replicates.

Where the sample had a 95 per cent. proportion of penicillin II, the data to be published would show that the errors found were very much less (less than ± 4 per cent.) for the main component.

Mr. STROUD asked if the quality of the ether used in the development of the strips was standard, and was the same quality always used? With respect to the iodimetric method of assay, the interference of unsaturated bonds is eliminated by the control blank experiment.

Dr. LEVI said they always used anaesthetic ether, but he did not think the quality was critical. It was, of course, essential for the ether to be damp.

Dr. E. C. WOOD considered that the necessary paper strips with clean-cut edges and precisely determined width could be easily made by the manufacturer. The makers of the pressure-sensitive adhesive tapes, so widely employed nowadays for sealing purposes, used a machine that should be quite capable of dealing with filter-paper. He would like to ask Mr. Robinson if he would venture a definition of the word "penicillin." The difficulty of defining the word "vitamin" was notorious, and it seemed that a similar difficulty was beginning to arise with the penicillins. If the definition were based on the chemical constitution, it would be difficult to exclude certain substances that have little or no anti-biotic activity. If, on the other hand, a functional definition was attempted, very careful wording would be required if the definition was to be neither too wide nor too narrow.

Mr. ROBINSON referred Dr. Wood to the Therapeutic Substances Amendment Regulations, 1946, in which a statutory definition of penicillin is given.

Mr. S. S. RANDALL asked how many antibiotics the authors had discovered. With similar technique, but different solvents and buffers and with the aid of "differential" organisms, he had found evidence of fourteen.

Dr. LEVI said they had found at least nine. In reply to another question he said that so far they had encountered no differences in results attributable to differences between *B. subtilis* strains.

Dr. HEATLEY asked if penicillin K was not still being called penicillin IV by some workers and if this error had ever been refuted in print.

Dr. LEVI said that they discovered penicillin IV during the war and did work on it which, of course, could not then be published. In the meantime Americans had been working on penicillin K and it had been assumed that the two were identical. But there could now be no doubt that they are in fact quite distinct.

Commenting on Dr. Heatley's question, Mr. ROBINSON said that penicillin K was probably a mixture of several penicillins, with pK values of about 5, and penicillin IV was probably not identical with any of them. Craig, in America, had designed an apparatus capable of partitioning penicillin a large number of times between an aqueous phase and a solvent phase, and the results had indicated the existence of numerous penicillins not yet characterised.

Mr. C. R. BOND, in congratulating the authors on the work they had done, said that the method had enabled a much clearer picture of the complex composition of penicillin as produced by the mould to be formed and was a valuable weapon in work on the purification of penicillin and the separation of individual penicillins. He asked the authors whether they had applied the method to streptomycin, and, if so, whether this was likely to prove as complex as penicillin. He also enquired whether they could provide any information on the nature of the side chain in the newer penicillins such as IV, VI and VII.

With reference to the confusion existing on the nomenclature of the penicillins, particularly penicillins K and IV, he said that the existence of more than one penicillin was first exclusively demonstrated in the I.C.I. laboratories in July, 1943, by the chromatographic separation of what were now known as penicillins I and II. At that time the constitution of penicillin was not known and it was suggested that penicillins as they were discovered should be distinguished by the use of Roman numerals. Penicillin III was discovered in January, 1944, and shown to be a *p*-hydroxy derivative of penicillin II. Penicillin IV was isolated shortly afterwards in the I.C.I. laboratories but sufficient was not obtained for a complete determination of its constitution. It appeared, however, to be closely related to penicillin I; it was certainly different from penicillin K, which was discovered later.

Dr. LEVI said he would like to see streptomycin put on strips. He knew nothing of the chemical constitution of penicillins IV, VI or VII. There was confusion between penicillins IV and K in America and in this country.

Mr. W. F. WILKINSON asked if differences in quality between different samples of agar had been found to have any effect on the readability and validity of results. In determinations of fungistatic and bacteriostatic values, considerable differences due to this cause had been encountered.

Dr. LEVI thought that might well be, but he had not experienced difficulties in that direction, except with one particular batch of agar, which gave unusually small zones.

Polarography

The following four papers were read at the Meeting of the Physical Methods Group on November 26th, 1946.

Amperometric Titration

By J. T. STOCK

POLAROGRAPHIC technique can be used not only for the direct determination of electro-reducible or electro-oxidisable substances in solution, but also for following the progress of a reaction such as the precipitation of one of the constituents of the solution. It is thus possible to employ polarometry for the location of the end-point in certain titrations. Since the process involves the measurement of current, Kolthoff and Pan[1] proposed the name *amperometric titration* to replace the term *polarometric titration* suggested earlier by Majer.[2] Though subject to some criticism,[3] the newer term appears to have become firmly established.

Titrations with the aid of the dropping mercury electrode were first performed in 1929 by Heyrovský and Berezicky,[4] who studied the precipitation of barium, strontium and calcium as the sulphate, carbonate, etc. These workers added successive measured volumes of the reagent, *e.g.*, potassium sulphate, to the solution to be titrated, *e.g.*, barium chloride, polarographed the latter after each addition and measured the height of the barium wave. The height decreased regularly as the reagent was added, owing to removal of electro-reducible barium ions from solution. After equivalence had been attained, further addition of the reagent caused the wave height decrease to continue at a smaller rate, owing to the suppression of barium ions by the solubility-product effect. The change in the rate of decrease was taken to indicate the end-point, although the latter was actually found by comparing the wave height with that of an artificial equivalence solution.

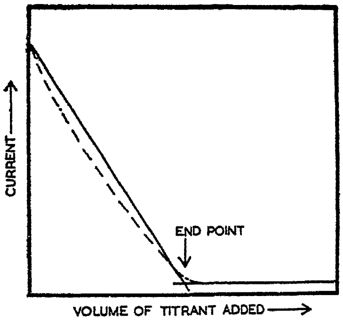


Fig. 1. Typical amperometric titration curve obtained when only the substance titrated is wave-forming.

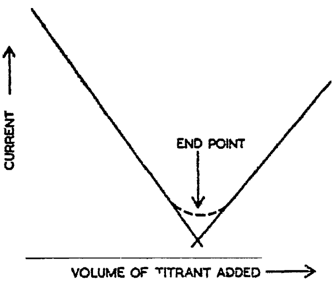


Fig. 2. Curve obtained when both reagent and substance to be determined are wave-forming.

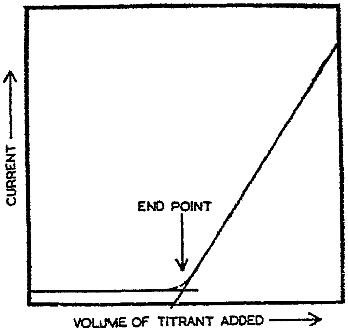


Fig. 3. Curve obtained when the reagent only is wave-forming.

Considerable advances were made by Majer,[2] who discussed the general characteristics of the method and pointed out the simplification, both in manipulation and in the necessary apparatus, which resulted from the use of a fixed applied e.m.f. instead of recording a series of polarograms. Several other Continental workers[5,6,7,8,9,10] have also contributed to the development of the method. The systematic investigations by Kolthoff and his co-workers, the first results of which were published in 1939,[1] have shown that amperometric titration is capable of high accuracy, and have done much to extend the applications of the method.

If a solution of an indifferent electrolyte, such as potassium nitrate, containing lead ions be placed in a polarographic cell and a potential of about —1·2 volt (all potentials are with respect to the saturated calomel electrode) be applied to the dropping mercury electrode, a diffusion current will be obtained, since lead ions are reduced well below this potential. (It is assumed that the usual precautions of oxygen-removal and of maximum-suppression have been observed.) Suppose small successive volumes of potassium oxalate solution are now added, the current being measured after each addition. Usually, a stream of hydrogen or nitrogen is bubbled through the solution for a few minutes to assist mixing; the gas stream is stopped before the current is read. The current flowing falls progressively as lead oxalate is precipitated, and becomes very small when equivalence is reached. Since oxalate is not reducible under the experimental conditions, its continued addition does not cause further change in the current. If the latter be plotted against the corresponding volume of oxalate solution added, a curve, shown diagrammatically in Fig. 1, consisting of two straight lines intersecting in the form of a rough "L" is obtained. The end-point volume of the titration is indicated by the point of intersection of the two branches of the curve.

In practice, introduction of the titrant unavoidably dilutes the test solution, causing deviation from linearity, as depicted by chain lines in Fig. 1. This effect may be minimised by using a titrant 10 to 20 times more concentrated than the test solution and in precise work may be allowed for by correcting the current readings by the relationship:

$$i_{\text{corrected}} = i_{\text{measured}} \times (V + v)/V,$$

where V is the initial volume of the test solution and v is the volume of reagent added.[1,6]

Owing to the solubility of the precipitate, a "rounding-off" in the region of the end-point, as shown by broken lines in Fig. 1, is observed. Except when the solubility is appreciable, or when extremely dilute solutions have to be titrated, this curvature does not interfere; the end-point is located by producing the linear portions of the branches to intersect. In most cases, therefore, it is sufficient to plot three or four readings on each side of the end-point and not too close to the latter and to draw through the points a pair of straight lines to intersect at the end-point volume.

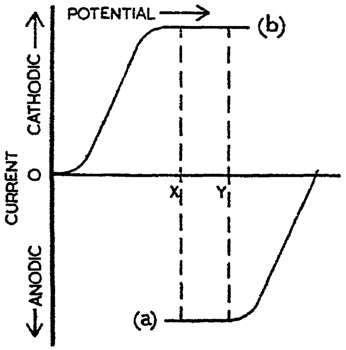


Fig. 4. Anodic and cathodic curves.

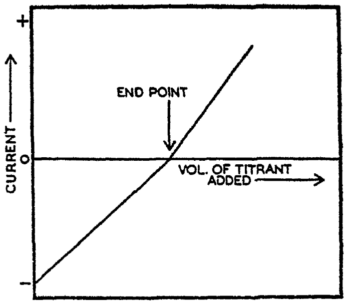


Fig. 5. Titration of an electro-oxidisable substance with a reducible reagent.

Suppose that the titration be repeated with potassium dichromate solution in place of the oxalate. As before, the current falls progressively until the end-point is reached. Since, however, dichromate forms a wave under the experimental conditions, continuance of the titration causes the current to rise progressively. The "V"-shaped titration curve (Fig. 2) obtained is characteristic of titrations in which both reagent and substance to be determined are electro-reducible.

If the titration with dichromate be performed at zero potential and with the lead-containing solution rendered slightly acid, the current, initially small, does not alter appreciably until the end-point has been reached. It then increases progressively as addition of dichromate is continued, giving a titration curve of the "reversed L" type shown in Fig. 3. Such curves are characteristic of titrations in which the reagent, but not the substance to be determined, is reducible.

The titration curves so far discussed resemble those obtained in conductometric titration, although quite different principles are involved. In both conductometric and amperometric techniques the method of finding the end-point does not involve readings near this region. Accordingly, both techniques are applicable to the titration of very dilute solutions, or in other circumstances when methods based on the actual recognition of the end-point fail. However, foreign or indifferent salts, traces of which often have to be guarded against in conductometric work, may be present if the amperometric technique is employed, and are in fact normally added.

The marked increase or cessation of flow of current that occurs in the end-point region of an amperometric titration is reminiscent of the effects observed in the "dead stop" method of titration.[12] Both methods involve polarisation phenomena, although of different kinds. However, no actual measurement of the current is made when the "dead stop" method is used, hence the term "amperometric" is not strictly applicable to it. Alternative terminology has been proposed by Guzman and Rancano.[13]

Another type of titration curve is obtained when the substance to be determined forms an *anodic* wave (*i.e.*, is *oxidised* at the dropping mercury electrode), whilst, under the same conditions, the titrant forms a cathodic wave. Such titrations were first studied by Strubl.[6] Thus in acidified tartrate solution tervalent titanium forms an *anodic* wave (Fig. 4*a*), the half wave potential of which is —0·44 volt. In the same medium tervalent iron gives a cathodic wave at a less negative potential, as depicted at *b* in Fig. 4. Between the potential limits X and Y, either substance yields its diffusion current. If, therefore, an acid tartrate solution containing titanous ions be electrolysed at a potential lying between X and Y and titrated with ferric chloride solution, the *anodic* current initially obtained decreases to zero as the titanous ions are oxidised by the titrant. Beyond the end-point, however, the ferric iron now present gives rise to a *cathodic* current, the magnitude of which increases as further additions of titrant are made. Thus a *reversal* of the current occurs; the titration curve is shown diagramatically in Fig. 5. Generally, the slopes of the portions of the curve before and after the end-point are not identical. In the reverse titration, *viz.*, when the titrant forms an anodic wave and the substance to be determined a cathodic wave, the titration curve slopes the opposite way. The work of Strubl was extended by Spalenka,[7] who studied the determination of chromate, ferricyanide, etc., by the titration of titanous chloride solution. Other titrations involving substances that form anodic waves are of halides with soluble salts of silver[14,15] or mercury,[16] of chromate with ferrous ammonium sulphate[17] and of stannous tin with copper sulphate.[18] The last example is interesting in being based upon *compensation* of the anodic current of tin by the cathodic current of copper, no direct reaction between the ions occurring in the bulk of the solution.[19]

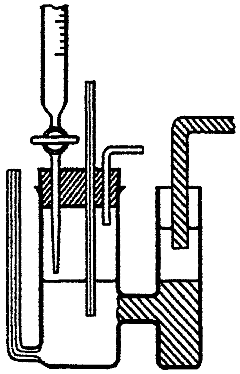


Fig. 6. Titration cell for use with external reference electrode.

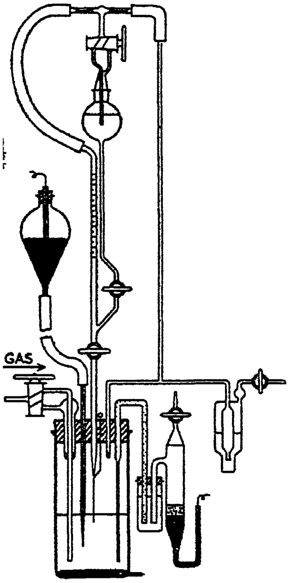


Fig. 7. Kolthoff and Langer titration assembly.

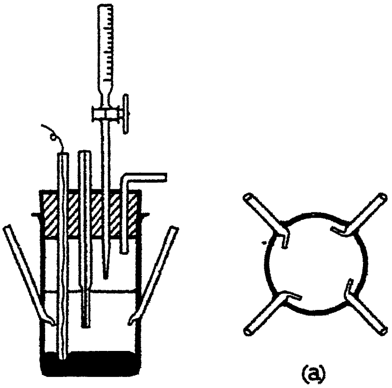


Fig. 8. Cell designed to promote efficient stirring.

Although most examples of amperometric titration hitherto investigated have been performed with the dropping mercury electrode, increasing interest is being shown in the possibilities of the rotating platinum micro-electrode.[17,20,21,22,23,45] This is especially suitable for titrations that can be carried out in an open beaker. In its simplest form the rotating electrode consists merely of a short piece of platinum wire sealed through the wall near the lower closed end of a length of glass tubing, which is rotated at constant speed.

Various forms of titration cell have been described. That shown in Fig. 6 is used in conjunction with an external reference electrode (usually a large saturated calomel electrode) an agar - potassium salt plug allowing connection to be made with the solution to be titrated.[14] Since the titrant may be stored and introduced into the cell in absence of air, the Kolthoff and Langer apparatus (Fig. 7) is suitable for routine work.[24] In a cell designed by Spalenka,[7] the gas stream enters through four tubes bent as shown at (a) in Fig. 8. Intensive stirring of the solution is thus caused. Owing to the danger of breaking fragile electrode capillaries, burette jets, and the like, on attempting removal from rubber stoppers, the writer prefers to use for beaker-type cells the bakelite clamp-on cap shown in Fig. 9. The electrode capillary or burette jet passes through a short glass tube cemented into the cap, a short sleeve of rubber tubing preventing ingress of air.[25] A tall-form 100-ml. lipless pyrex beaker A is used as the cell, and is retained by stirrup B. The thumbscrews CC enable the lip of the beaker to be lightly pressed into rubber joint D, thus forming a gas-tight seal.

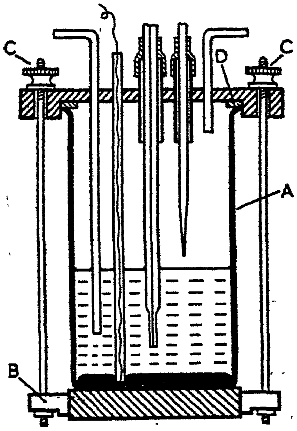


Fig. 9. Beaker-type cell with clamp-on cap.

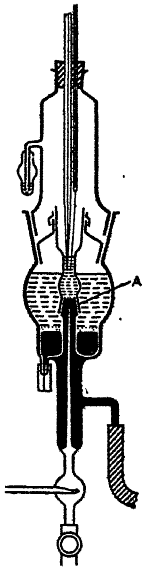


Fig. 10. Micro-cell incorporating calomel electrode.

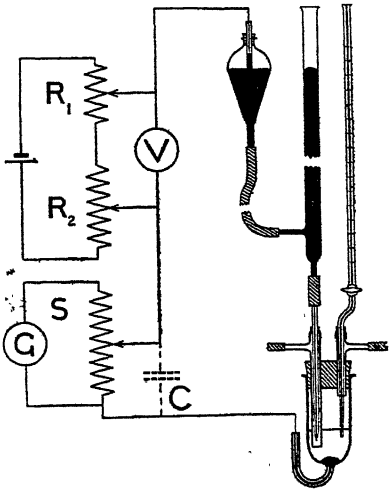


Fig. 11. Circuit arrangement for amperometric titration.

Langer has described both macro- and micro-scale cells.[26] The latter is depicted in Fig. 10. It incorporates a saturated calomel reference electrode which surrounds the titration chamber, junction with the test solution being made through ground joint A. Other cells for the titration of small volumes of solution have also been described.[27,28] Since electrical measurements are made only as a means of end-point location, thermostatic control, which is at least desirable in normal polarography, is unnecessary. For the same reason the dropping mercury electrode capillary need not be calibrated, so replacement is simple if a breakage occurs.

Amperometric titration is normally carried out with an applied e.m.f. that remains constant during the entire operation. Hence the electrical apparatus is comparatively simple. The potentiometer device shown in Fig. 11 may be used to select the desired applied e.m.f., the value of which is indicated by voltmeter V. To measure the current, a mirror galvanometer G, or, if available, a sensitive microammeter, is used. Shunt S permits adjustment of the sensitivity. To damp the galvanometer oscillations, a large-capacity electrolytic condenser C may be incorporated in the circuit.[8,29,30] A compact polarising unit that permits the degree of damping to be adjusted has been described by the author.[28]

Amperometric titrations involving lead have attracted considerable attention. Titration of this metal with potassium dichromate yields very accurate results in solutions as dilute as 0·001 *M*. As indicated above, dichromate in acid solution is reduced at zero potential. The titration may therefore be performed without the aid of a battery or polarising unit, the dropping electrode being merely short-circuited through the galvanometer to the reference electrode.

Several anions may be titrated with lead nitrate solution. The titration of sulphate, first studied by Majer,[2] has attracted particular attention. A titration curve of the type shown in Fig. 3 is obtained, and, since lead sulphate is appreciably soluble, considerable rounding of the curve occurs. In an attempt to overcome this, Majer evolved a somewhat elaborate graphical method for locating the end-point. Other mathematical studies upon the location of the end-point in titrations involving the formation of appreciably soluble precipitates have also been made.[31,32] To reduce the solubility of lead sulphate, Spalenka[7] added ethanol to the solution before titration; this procedure was extensively studied by Kolthoff and Pan.[11] After oxidation to sulphate, sulphur in coke may be determined in this manner.[47] The determination of sulphate in precipitated alumina presents difficulties owing to the solubility of lead sulphate in aluminium nitrate solutions.[33] However, by adjusting the *p*H, keeping the aluminium content down and adding ethanol, satisfactory results may be obtained.

The amperometric titration of chloride with lead nitrate solution is also rendered possible by addition of ethanol to the solution.[34] The alcoholic content at the end-point should be 55 to 60 per cent. According to Thanheiser and Willems,[10] molybdate may also be determined by titration with lead nitrate solution, and the method may be used for the determination of molybdenum in steels. In the latter instance, iron, and, if present, tungsten must first be removed. Lead nitrate solution may also be used for the titration of ferrocyanide and of oxalate.[11] Another reagent for amperometric titration of ferrocyanide is zinc chloride solution. Langer showed that solutions that are from 0·01 *N* to 0·001 *N* with respect to fluoride can be titrated with thorium or lanthanum nitrate solution, the accuracy being about 0·5 per cent. A potential of −1·7 volt is employed. Fluoride forms no wave, so that a "reversed L" titration curve is obtained.[39] In the titration of phosphate with bismuth oxyperchlorate solution, described by Neuberger,[8] a curve of the same type is obtained. The method was applied to the examination of superphosphate. Even with appreciable amounts of phosphate, results are not very accurate. A much more satisfactory reagent for this anion is uranyl acetate.[36]

In addition to precipitation as sulphate,[2,4] barium in neutral solution may also be determined by titration with potassium chromate solution.[1] If lead is present in addition to barium, the former is precipitated first, since the solubility product of lead chromate is much less than that of barium chromate. If the titration is performed at a potential of −1·0 volt, lead and chromate form waves, but barium does not. Hence as titration proceeds, the current at first falls progressively until precipitation of lead is complete; it then remains at almost zero until sufficient chromate has been added to precipitate the barium. Further addition of reagent causes a progressive rise in current. A titration curve of the type shown in Fig. 12, consisting of *three* linear portions, is thus obtained, permitting the simultaneous determination of lead and barium. Though the second point of intersection corresponds correctly to the complete precipitation of lead and barium, co-precipitation causes the first to occur considerably after the theoretical end-point for lead. The solubility product of copper quinaldinate is much lower than that of the corresponding zinc salt,[37] so that the simultaneous titration of copper and zinc with quinaldinic acid is similarly possible.[38]

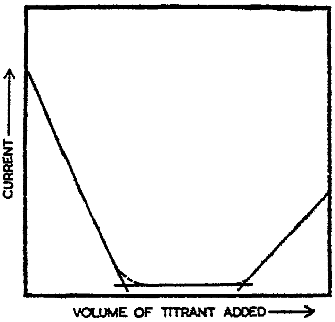
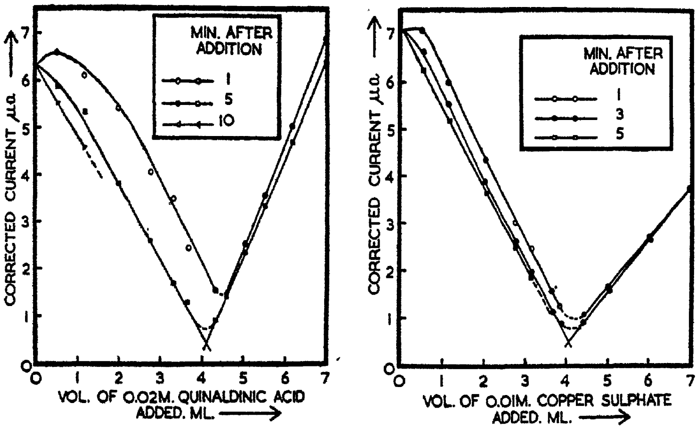


Fig. 12. Titration of two substances in the same solution.

Many of the specific organic reagents for metals are polarographically reducible and have been used successfully in amperometric titration. According to Neuberger,[8] Abresch showed that dimethylglyoxime could be used for the titration of nickel. Little detail is given in Neuberger's paper. The systematic studies of Kolthoff and Langer[24] showed that highly satisfactory results could be obtained, and these workers showed that the method was applicable to the determination of nickel in steels. Copper may be accurately titrated either with α-benzoinoxime[39] or with α-nitroso-β-naphthol solution.[40] The latter reagent is also suitable for the determination of cobalt and palladium. Copper may also be determined by means of salicylaldoxime solution.[9] Excess of the latter is added to the solution to be examined and the determination is completed by titrating back with standard copper solution. Reversal of titration or employment of back-titration is sometimes useful in reactions in which precipitation is sluggish. This is demonstrated by the curves shown in Fig. 13. Those at (*a*) were obtained by adding 4 ml. of 0·01 *M* copper sulphate to 40 ml. of an acetate buffer of *p*H 4·9 and titrating with 0·02 *M* quinaldinic acid solution. If 4 ml. of the latter are diluted with 40 ml. of the buffer and titrated with 0·01 *M* copper sulphate, titration curves as at (*b*) are obtained. These show that equilibrium is almost obtained within 3 minutes of mixing; when quinaldinic acid is added to the copper-containing solution, attainment of equilibrium takes several times as long.[38]

For the titration of bismuth, 8-hydroxyquinoline has been used.[41] According to Zanko,[42] this reagent may also be used to titrate zinc, copper and aluminium.



(*a*) (*b*)

Fig. 13.

(*a*) Titration of copper with quinaldinic acid. (*b*) Reverse titration.

The amperometric titration of α-tocopherol with gold chloride solution is interesting as an example of the determination of an organic substance with an inorganic reagent.[43] In a similar manner, silver nitrate solution may be used for the titration of mercaptans.[21,45] A titration in which both reactants are organic in nature is that of picrolonic acid with methylene blue, which has been used as an indirect method of determining calcium.[44]

Since the principles involved are mainly as in polarography, amperometric titration possesses most of the advantages and disadvantages of the polarographic method. Interference by metals or other substances that form waves at potentials below that at which the particular titration has to be performed is serious. Although use of a counter-current device[29,46] may minimise such interference, the presence of high concentrations of such substances renders titration impossible. By introduction of nitro, azo or other readily reducible groups it might be possible to modify existing specific organic precipitants by rendering them wave-forming and hence usable at low potentials The likelihood of interference would then be diminished.

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L.C.C. NORWOOD TECHNICAL INSTITUTE
LONDON, S.E.27

August, 1946

DISCUSSION

MR. A. C. MASON asked whether, with precipitation reactions that take some minutes to reach completion, the method would not require undue time for a determination requiring the plotting of several points.

DR. W. CULE DAVIES asked what were the advantages of amperometric titrations over other well known methods of titration. Possibility of titrating very dilute solutions seemed to be one.

MR. J. HASLAM asked what order of accuracy should be expected when two points before and two points after the end-point were tested.

DR. W. STROSS asked how long an amperometric titration took to perform.

MR. K. GOLDSCHMIDT asked if the author had any information concerning the application of the method to very concentrated solutions. Was it a fact that trouble rather difficult to eliminate occurred?

MR. F. C. J. POULTON asked if Mr. Stock had experience of amperometric titrations of copper at very low concentrations, of the order of $M/20,000$, with α-benzoin oxime. Could he say if in such instances the amperometric method had any advantages over the orthodox polarographic method?

MR. W. FURNESS asked if Mr. Stock would amplify his statement of the conditions that govern the choice of those points at which tests should be made. If a very insoluble salt is produced it would seem best to choose points close to the point of discontinuity of the curve; but when the salt produced had appreciable solubility points well removed from the equivalence point would seem preferable.

MR. STOCK, replying to Mr. Mason, said that with the organic precipitants used 2 or 3 minutes for completion of precipitation could be allowed and sometimes less at each point. The total time required for a titration was normally less than half an hour and often twenty minutes were sufficient. In reply to Dr. Cule Davies he said that another advantage of the method was that one did not have to ascertain the end-point directly. The method was comparatively new and other advantages might appear later. In reply to Mr. Haslam he considered that if the selected points were well chosen an accuracy rather better than 1 per cent. should be obtained, especially if a correction was made for dilution by the titrant. He had no information about application of the method to very concentrated solutions. In reply to Mr. Poulton the stock copper concentration he had titrated with α-benzoin oxime was $M/1000$; he had titrated 5 μg. in 500,000, but such titrations were difficult. In reply to Mr. Furness he said that normally he tested three points on either side of the end-point, to make sure that they lay on a straight line. With soluble precipitates the matter was more complicated, and several mathematical papers on the location of the end-point had been published, notably one by Langer and Stevenson.

The Rotating Platinum Electrode

By C. J. O. R. MORRIS

The dropping mercury electrode has proved so satisfactory for most purposes in polarographic analysis that there has been little incentive to search for a substitute, at least so far as cathodic reactions are concerned. There are, however, two classes of reaction for which an alternative electrode system would be valuable. First, those cathodic reductions in which mercury interferes chemically with the electrode process, and secondly in the study of anodic oxidations. The latter class has not been of very much importance in inorganic polarography, but the increasing interest in the polarographic analysis of organic compounds has more recently caused greater attention to be given to such processes. There is, however, an important limitation in the study of anodic oxidations with the dropping mercury electrode. While it is possible to carry out cathodic reductions at potentials up to about $-2{\cdot}7$ v., on the anodic side oxidation of the mercury itself limits the range to about $+0{\cdot}4$ v. The use of an inert electrode would extend the range up to about $+1{\cdot}1$ v., at which point evolution of oxygen from the anode sets the upper limit of the process.

Many workers have used solid metallic electrodes in the study of diffusion currents, both cathodic and anodic, but obstacles such as electrode polarisation (as distinct from concentration polarisation) made it very difficult to obtain reproducible results, and it was not until the introduction of the dropping mercury electrode by Heyrovský that the measurement of diffusion currents became sufficiently reliable to be of value to the analyst. Nevertheless, under strictly controlled conditions, quantitative measurements can be made with solid metallic electrodes. Thus Glasstone and Reynolds[1] studied the oxidation of ferrous ions at a platinum anode, and showed that the diffusion current was proportional to the ferrous ion concentration.

The first thorough investigation of the platinum electrode from the standpoint of the analytical chemist was carried out by Laitinen and Kolthoff.[2,3] They first examined the stationary platinum electrode and derived an equation for the diffusion current, *viz.*, $i = knDC$, in which i is the diffusion current, n the number of electrons involved in the electrode reaction, D the diffusion coefficient, C the molar concentration of the diffusing substance, and k a constant. They confirmed this equation for silver, copper, thallous, lead, ferric and ferrous ions, and showed that analyses could be carried out with an accuracy of about 1 per cent. provided proper precautions were taken. In particular it was essential to prevent thermal convection near the electrode, and also to ensure absence of mechanical vibration. They found that the temperature coefficient of the diffusion current was about 4 per cent. per degree C., as compared with about 2 per cent. for the dropping mercury electrode.

The stationary platinum electrode has, however, certain disadvantages. The greatest of these is the necessity of waiting for at least 2 minutes at each value of the applied potential for equilibrium to be reached. Thus a polarographic record in the usual sense is impossible. Other disadvantages are the rigorous temperature control necessary and the possibility of electrode polarisation.

Laitinen and Kolthoff found that these difficulties could be largely overcome by rotating the platinum electrode at a constant rate. The zone of convection equilibrium attained with the stationary platinum electrode was then replaced by a zone of uniform stirring. Owing to the much greater rate of diffusion in this zone, the current density was much higher, and the observed diffusion currents were 10 to 20 times greater. In fact, under comparable conditions, the rotating platinum electrode gave larger diffusion currents than the dropping mercury electrode. The rotating electrode was less sensitive to external conditions than the stationary electrode, showed a temperature coefficient of diffusion current of about 2 per cent. per degree C. in the reactions studied, and was in general more similar in its behaviour to the dropping mercury electrode. It was found difficult, however, to get reproducible results from day to day, and this has also been our own experience with this electrode. Laitinen and Kolthoff suggested therefore that perhaps the greatest utility of the rotating platinum electrode was as an indicator electrode in amperometric titrations, where such reproducibility is not essential. Kolthoff and his collaborators have reported several such amperometric titrations, an interesting example being the titration of –SH groups with silver nitrate solution (Kolthoff and Harris[5]). The type of electrode used by Laitinen and Kolthoff is shown in Fig. 1. Kolthoff and Harris have suggested a modified form for use in suspensions (Fig. 2). This apparently functions by minimising disturbances of the diffusion layer by solid particles

A comparison of various electrodes for the continuous estimation of oxygen in sea-water was carried out by Giguere and Lauzier.[7] They examined the dropping mercury electrode, the stationary platinum electrode and the rotating platinum electrode as cathodes, with the mercury pool, the saturated calomel electrode and various metallic electrodes as anodes. They made the important observation that when platinum electrodes were used for long periods with either the mercury pool or the saturated calomel electrode as anode, mercury was deposited electrolytically on the cathode and led to erratic results. They were able to overcome this difficulty by surrounding the anode with a fine-mesh platinum wire cage maintained at —0·2 v. with respect to the anode Their apparatus is shown in Fig. 3 They found that the rotating platinum electrode gave considerably larger diffusion currents than either the stationary platinum electrode or the dropping mercury electrode, and obtained a linear relation between diffusion current and oxygen concentration over a wide range. The work of Giguere and Lauzier would appear to have wide application, especially in the measurement of oxygen uptake in biological systems, for theoretically the polarographic method should be more sensitive than the manometric methods generally used, and it offers the additional advantage of a continuous record.

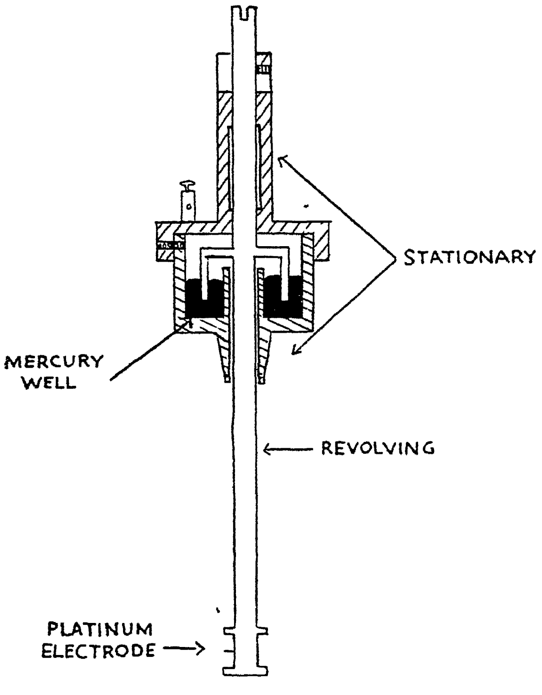

Fig. 1.

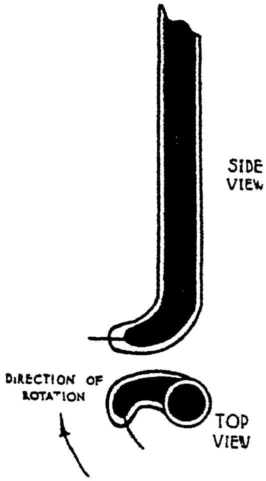

Fig. 2
(Reproduced from *Ind. Eng Chem , Anal Ed* , 1946, **18**, 162).

An interesting attempt to overcome one of the most serious disadvantages of solid electrodes, *viz.*, electrode polarisation, has been described by S. D. Müller.[8] His apparatus is shown in Fig. 4. Two platinum electrodes are used with either a mercury pool or a solid metallic anode. When one electrode is cathodically polarised, the other is kept at anode potential. The states of polarisation and depolarisation are reversed at a constant frequency by means of a commutator. Müller used a metronome device to actuate the commutator and used polarisation times of 0·3 to 5·0 secs.; the best results were obtained at 1 to 2 secs. He examined the cathodic reduction of copper, lead, cadmium and zinc in sodium potassium tartrate solution and his published polarograms appear very similar to parallel runs with the dropping mercury electrode. We are constructing in our laboratory a similar arrangement with electronic control of the polarisation period. This is shown in Fig. 5. The frequency is controlled by the neon tube relaxation oscillator, which operates a uniselector switch acting as commutator. We are hoping to study various organic anodic oxidations with this apparatus.

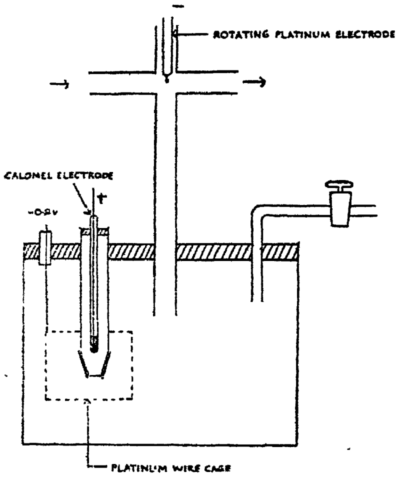

Fig. 3.

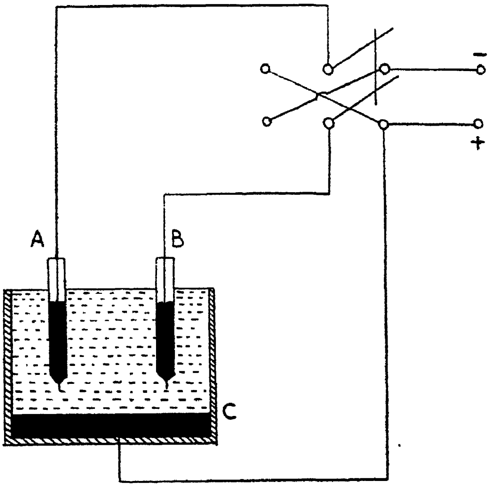

Fig. 4.

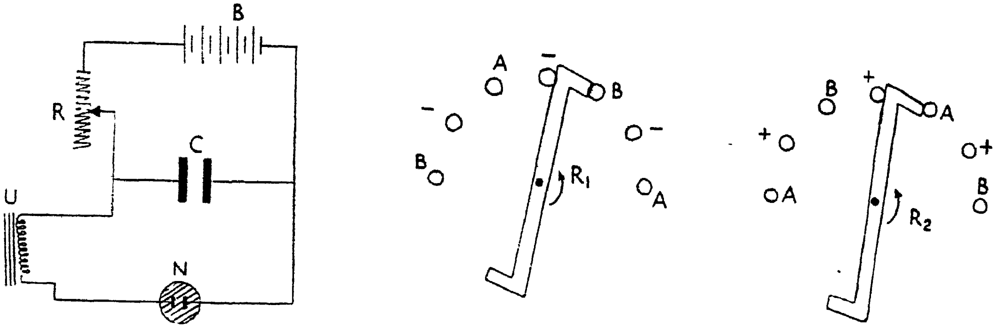

Fig. 5

The various types of metallic electrode described do not replace the dropping mercury electrode, which is still the most reliable for general analytical purposes. They are, however, of value in special circumstances, and further study of such electrode systems will doubtless extend still further the range of polarographic analysis.

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DISCUSSION

Mr. J. T. STOCK asked if, in the Müller double electrode system there was a pronounced temperature coefficient, as might be expected from Dr. Morris's remarks about stationary micro-electrodes in general, for in the fundamental equation for such electrodes the diffusion current appears as the first power and not as the square root.

Dr. J. E. PAGE asked if Dr. Morris had used the rotating platinum electrode to study the oxidation of organic substances, and if he knew whether its application to the measurement of oxygen concentrations had been used in respiration experiments (*cf.* R. J. Winzler, *J. Cell. and Comp. Physiol.*, 1943, **21**, 229).

Dr. J. E. B. RANDLES asked if any phenomena similar to those appearing with the ordinary direct mechanism had been noted. He had thought that Muller used the ordinary galvanometer for his recording.

Dr. W. STROSS mentioned two arrangements described in a doctorate-thesis by W. Kaeppel (printed at Bonn in 1941) from v. Stackelberg's laboratory; these were not accessible in ordinary publications but might be of interest. The first consisted of four stationary electrodes dipping in the test liquid which was separated by a diaphragm from a calomel reference electrode. A rotating contact connected one electrode at a time in rapid succession (5 to 20 cycles per potential increase of 0·1 volt) as the cathode, whilst the other three were connected with a depolarising rail to which various potentials could be applied by means of a battery and a variable resistance. The second apparatus also consisted of four electrodes of which in turn one is dipped into the solution by an electromagnet every second, whilst the other three are kept above the liquid. Both instruments were used for experiments on thiourea.

Dr. PAGE said that Dr. Kaeppel's thesis contained a number of interesting points. The quinone - hydroquinone system was regarded as reversible but when it was examined by Dr. Kaeppel's technique oxidation of hydroquinone and reduction of benzoquinone occurred at different potentials. Some of the statements in the thesis were unexpected and required confirmation.

Dr. ZADE asked if it would be possible to use alternating current in amperometric titration with the rotating platinum electrode; if so, contact trouble could be overcome by capacitive coupling.

Mr. G. S. SMITH asked if in order to avoid difficulty with contacts it would be possible either to cause the electrode to make a circular movement in the liquid without rotating or to make the liquid rotate round a stationary electrode.

Dr. MORRIS, in reply to Mr. Stock, said he thought that the Müller system might have a relatively high temperature coefficient, but he imagined trouble due to convection would be reduced. In reply to Dr. Page, he said he knew of no published work on the study of oxidation of organic compounds by means of the rotating platinum electrode; he and his colleagues were mainly interested to applying it to biological analysis. Replying to Dr. Randles, he said that contact trouble was the main difficulty with the rotating electrode, but it could be overcome; the kind of galvanometer used by Müller was not clear from his reference to it. To Dr. Zade's question as to the possibility of using alternating current he replied that this could be done. He thought Mr. Smith's suggestions for avoiding contact difficulties were worth trying. Possibly, however, he had made too much of contact trouble ; there were ways of overcoming it. Rotation of the liquid by means of a solenoid had been tried but was not a success.

The Application of the Cathode Ray Oscillograph to Polarography: Underlying Principles

BY J. E. B. RANDLES

THE ordinary polarograph is applicable to the estimation of small quantities of many metals and electro-reducible or -oxidisable substances in general. Its use frequently gives results more accurate than are easily obtainable by purely chemical means and very often in a mere fraction of the time required for a chemical analysis. The most usual form of commercial instrument records the current - voltage curves photographically. This has some disadvantages in that an appreciable amount of time is spent in recording, developing and drying the polarograms before they can be measured. Furthermore, the actual form of the polarogram cannot be seen until the photograph is developed, so that any malformation is not perceived until the recording, or recordings, are completed. The photographic procedures are eliminated in pen recording instruments now made, and the polarogram can be seen as it is recorded, but the recording is still a comparatively slow process. Thus the idea of obtaining a current - voltage curve rapidly traced on the screen of a cathode ray tube has arisen. Work with this object in view has been carried out by Matheson and Nichols[1] and on less direct lines by Müller, Garman, Droz and Petras[2] and Heyrovský and Forejt.[3] However, in no case has any near approach to a practicable precise analytical instrument been attained.

It is believed that the apparatus described below represents a considerable development in this direction.

The basic principle of polarography is the measurement of the diffusion current passed by a micro-electrode in a state of concentration polarisation. It is usual to obtain, by one of two methods, a diffusion current, either steady or of steady average value, suitable for measurement by a galvanometer. The two methods are the use of the rotating platinum electrode and the use of the dropping mercury electrode. If, on the other hand, a cathode ray oscillograph is employed, rapidly changing currents may be followed without difficulty. This permits the measurement of diffusion currents under quite different conditions. To explain this difference of principle a brief consideration of the elementary theory of diffusion currents is necessary.

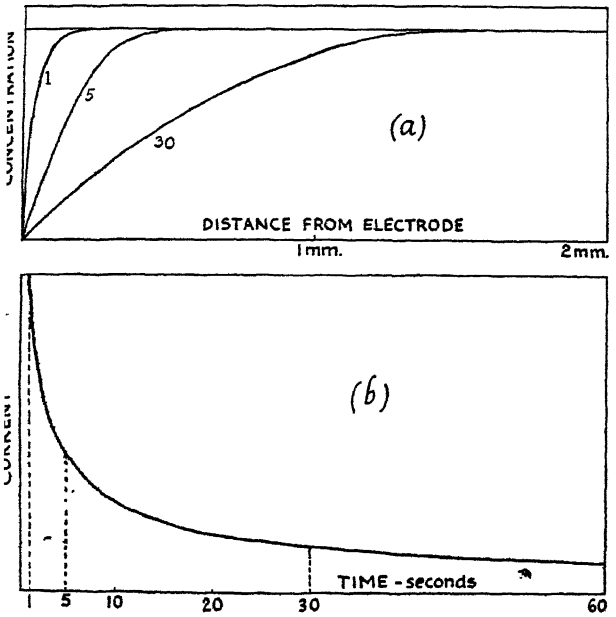


Fig. 1.

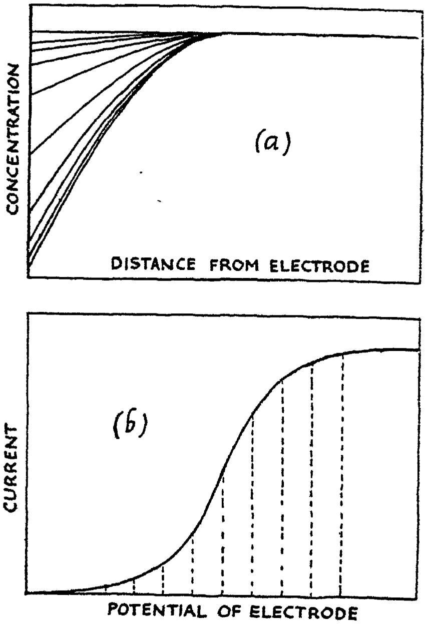
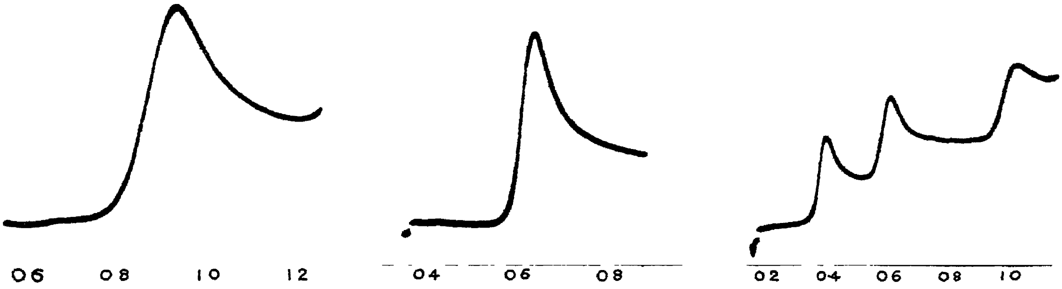


Fig. 2.

If a micro-electrode is immersed in a solution containing an electro-reducible or -oxidisable substance (and an indifferent electrolyte), and a potential, sufficient to cause reaction of the substance, is applied suddenly to the electrode, a diffusion current is obtained which changes with time as shown in Fig. 1 (*b*). The current starts at a high value and declines rapidly, being in its early stages proportional to $t^{-1/2}$, t being the time measured from the application of the electrolysing potential. This decline is due to the widening of the diffusion layer as electrolysis proceeds, which is illustrated in Fig. 1 (*a*). Ordinates are concentrations of the reacting substance in the solution and abscissae the distances from the electrode surface. Successive curves refer to the values of t shown, but the numerical values in general are only intended to show approximate magnitude. Since the current passed by the electrode is proportional to the rate of arrival of reacting substance at its surface, which is proportional to the concentration gradient of the substance, close to this surface, the reason for the decline of the current is evident from Fig. 1, (*a*).

Although the decrease of the diffusion current to a stationary electrode becomes slower as time goes on, the current becomes unsteady owing to convection effects resulting from the concentration differences near the electrode. A stationary micro-electrode, cannot, therefore, be used in conjunction with a galvanometer for polarographic purposes. The difficulty of the widening diffusion layer is ordinarily overcome in two ways, as already mentioned. The thickness of the layer may be limited by controlled stirring, as with the rotating platinum electrode. Or the electrode and the solution undergoing electrolysis may be automatically renewed at frequent intervals, as is in fact done when the dropping mercury electrode is used. The rotating electrode gives a steady current, and the dropping electrode a fluctuating current, but of constant average value, for a given potential of the electrode. The rate at which the potential of such an electrode is changed in order to obtain a current - voltage curve (*i.e.* the ordinary polarogram) must be slow (about 1·0 volt in 5 minutes) so that there shall not be significant lag in the attainment of the true current for each potential, and in the response of the galvanometer to the changes in current. Particularly the latter applies when the dropping electrode is employed, as the galvanometer must be sufficiently damped for it not to respond appreciably to the fluctuations of current with the growth and fall of each mercury drop. The form of current - voltage curve obtained with slow change of potential of the electrode, and the condition of steady, or steady average, current for any given electrode potential, is that of the ordinary polarogram. The gradual increase of current is due to the gradual decrease of the concentration of the reacting substance at the electrode surface as the reaction becomes more complete with the change of the potential of the electrode. This is illustrated diagrammatically in Figs. 2 (*a*) and 2 (*b*) for the case of a diffusion layer of constant thickness as at the rotating platinum electrode. The concentration - distance curves in Fig. 2 (*a*) correspond to potentials of the electrode indicated by dotted lines in Fig. 2 (*b*).

We may now turn to a consideration of diffusion currents varying with time as well as with the potential of the electrode. These arise, as we have already seen, from the sudden application of a suitable potential to a micro-electrode in a solution containing an electro-reducible or -oxidisable substance; or preferably, in practice, from the application to such an electrode of a rapid potential "sweep" covering say 1·0 volt in 1 to 2 seconds. Any electro-reduction or -oxidation having a polarographic "half-wave potential" within this range gives rise to a diffusion current which rises rapidly to a peak and then declines as the diffusion layer widens. Fig. 3, (*a*) is reproduced from a photograph of a cathode ray trace showing the current - voltage curve obtained with a platinum micro-electrode in this way. In practice successive potential "sweeps" are applied to the electrode, so that the trace is repeated, but with a stationary electrode there must normally be an interval of at least 10 seconds with the electrode at its starting potential, between the "sweeps," to allow time for the surrounding solution to regain its original uniformity.



(*a*) (*b*) (*c*)

Fig. 3 Current voltage curves. Abcissae are voltages of the micro-electrode negative to a silver - silver chloride electrode.

(*a*) 1/8 × $10^{-3}M$ $Cd^{\cdot\cdot}$ in *M* KCl. Stationary platinum electrode.
(*b*) 1/4 × $10^{-3}M$ $Cd^{\cdot\cdot}$ in *M* KCl. Dropping mercury electrode
(*c*) 1/16 × $10^{-3}M$ $Pb^{\cdot\cdot}$, $Cd^{\cdot\cdot}$ and $Zn^{\cdot\cdot}$ in *M* KCl. Dropping mercury electrode.

Although a stationary electrode has some advantages, in many cases a dropping mercury electrode is preferable. A potential "sweep" applied to a dropping electrode at a late stage of drop growth produces a current - voltage curve very similar to that obtained with a stationary electrode (see Fig. 3). The repetition of the "sweep" must be synchronised with drop formation in such a way that each sweep occurs at the same stage of drop growth, otherwise successive current - voltage curves would not be identical. The method of carrying this out will be explained shortly.

The potential "sweep" is provided by the charging of a condenser. Fig. 4 shows the simplest form of circuit for this purpose; it is very similar to one used by Matheson and Nichols. The starting potential of the "sweep" is controlled by potentiometer Q, and the rate of change of potential by rheostat R_2. The potential differences between points A and B and between C and D, are amplified by direct-current amplifiers and applied to the deflector plates of the cathode ray tube giving horizontal and vertical deflection of the beam, respectively. Horizontal deflection thus gives a measure of the potential difference across the polarographic cell and vertical deflection a measure of the current passing through it. The trace is therefore a current - voltage curve. The potential "sweep" is ended by short-circuiting the condenser by means of a relay-switch K. This relay is operated by an electronic circuit ("flip-flop" circuit) triggered by an impulse derived from the amplifier whose input is the potential difference across R_1. The impulse arises from the sudden change in current when the mercury drop falls. After a certain delay (controlled by a rheostat in the "flip-flop" circuit) to allow drop growth, the relay reopens and another potential sweep is started, to be terminated when the drop falls.

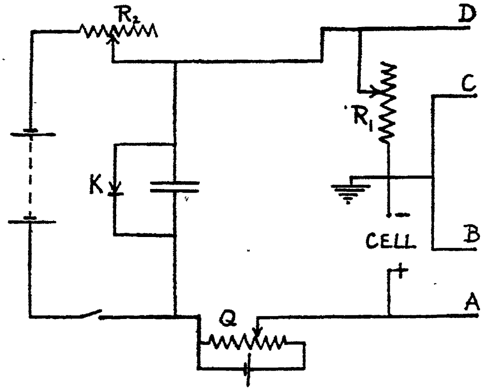


Fig. 4

In order that there shall be accurate proportionality between peak current and concentration of the reacting substance in the solution, not only is exact timing of the potential sweep required but also the rate of change of potential of the electrode must be constant and independent of the current passed. This cannot be achieved by the simple circuit of Fig. 4, for the rate of change of potential difference across the cell is inevitably affected by the sudden increase in potential drop in R_1 when the current rises. To overcome this an electronic circuit was devised which applies the potential difference to the cell in such a way that it is independent of the potential drop in R_1 and of the current. This, and the other circuits, will be described in detail elsewhere.[4]

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CHEMISTRY DEPARTMENT
BIRMINGHAM UNIVERSITY

The Application of the Cathode Ray Oscillograph to Polarography: General Lay-out and Uses of the Cathode Ray Polarograph

BY L. AIREY

IN the present paper the apparatus of which the principles have been explained by Mr. Randles in the preceding paper is considered in more detail from the standpoint of instrumentation and uses. It is emphasised that a practical Cathode Ray Polarograph has been designed and built,* having uses both in analysis and in research. Details of construction and circuits will be published elsewhere but the general lay-out of the main parts is indicated in block diagram form in Fig. 1 and further information on them is given in the following Sections 1 to 4.

1. *Cathode Ray Tube and Power Supply*—The tube itself has a fluorescent screen showing long afterglow properties (about 10 secs.). As will be seen from the diagram, the units are separate, but they may commercially be combined in one box.

2. *Amplifiers and Voltage Sweep Circuit, etc.*—These units are mounted side by side on a common chassis and housed in one box. The lay-out of the circuits follows conventional electronic practice but emphasis must be laid on adequate electrical screening of the input leads, etc., and the provision of a satisfactory anti-vibration support for the two valves in the voltage sweep circuit.

Other circuits included on the same chassis are (*a*) an electronic time delay circuit, (*b*) an auxiliary trigger amplifier and (*c*) an electromagnetic relay to control the cathode ray

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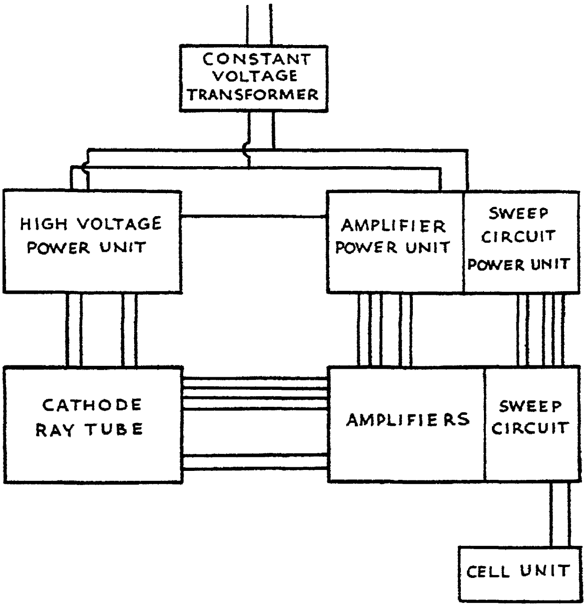
* The instrument was demonstrated at the meeting.

"spot" brightness during the quiescent condition. Circuit (*a*) is the conventional "flip-flop," with provision for converting it to a multivibrator. As the former, it consists simply of a circuit having one stable and one metastable state into which it can be thrown on receipt of a suitable impulse (from the amplifier); the duration of its stay in the metastable condition can be altered at will by variation of resistance values in circuit. As the multivibrator, the circuit possesses two metastable states, and the frequency of change from one to the other may be precisely governed by variation of resistance values.



In order to utilise to full advantage the long afterglow screen of the cathode ray tube, it is necessary that full spot brilliancy be employed. However, if the beam at this intensity should rest on one portion of the screen for any length of time permanent damage is done to the screen. To obviate this, the circuit (*c*) is introduced whereby the intensity is diminished during the quiescent part of the cycle.

3. *Power Supplies for Amplifiers, etc.*—Two independent power supplies for the amplifiers and voltage sweep circuit respectively are required. Good stabilisation by means of gas discharge tubes is necessary, and for the supply for the voltage sweep circuit additional resistance capacity decoupling after the stabilisers is desirable. The precise location of the power pack chassis with respect to the amplifiers is not important but it is desirable to keep the connecting leads as short as possible and to avoid stray alternating magnetic fields.

4. *Cell Assembly and Constant Voltage Transformer*—The cell assembly can be of any design whatsoever; the mercury column is earthed and the leads to the amplifier are electrostatically screened. A point of some importance is to ensure that the cell assembly is completely clear of stray magnetic fields from the apparatus. The constant voltage transformer is necessary only where main voltage fluctuations are greater than ±2 per cent.

As an analytical tool the apparatus is essentially a comparison instrument and it resembles other types of polarographs in requiring calibration with known standards. It is therefore essential that the controls shall be precisely reproducible in setting. It will be convenient to consider the sequence of operations required for the production of a quantitative polarogram.

(1) *Adjustment of the drop time*—With the capillary dropping in the test solution, the multivibrator circuit is set for a repetition frequency of 7 secs. (a convenient arbitrary time) With a constant known potential applied to the cell the drop break-away is apparent as a small "blip" in the cathode ray trace. By adjusting the head of mercury, the "blip" may be obtained at the same place on the trace in each successive cycle, and then the drop time is exactly that of the multivibrator. It is important, of course, that these measurements are made at a known potential, obtainable from a calibrated potentiometer included in the circuit.

(2) *Measurement of "peak"*—The voltage sweep rate is adjusted by means of the appropriate control. The multivibrator is switched over to "time delay" and the extent of the delay varied until a polarographic peak appears on the screen. Small variations in the fine control on the time delay circuit are then made until the horizontal projection of the "tail" of the peak is some convenient arbitrary length. A note is made of the initial starting voltage and of the "horizontal" amplifier gain setting. Finally the cell series resistor, R, is varied until the peak height is some definite figure (say 7·5 cm.). The value of R is recorded. The solution is then replaced by a standard, the peak length adjusted if necessary, and R varied to produce a peak height of 7·5 cm. The solution concentration being inversely proportional to the resistance, the unknown concentration is then easily evaluated.

The description just given applies to the reduction of a single species of ion. When more than one is present in solution, the technique is modified slightly. By careful use of the controls governing the starting potential across the cell, together with those of the time delay circuit, it is possible to isolate and measure, as described above, peaks corresponding to each individual ion. The method is advantageous in the estimation of a small amount of, say, cadmium (—0·6 v. versus S.C.E.) in presence of a relatively large amount of lead (—0·4 v. versus S.C.E.). By beginning at —0·5 v., no peak is obtained for the lead ion, and it is possible to estimate cadmium in presence of 50 times as much lead and possibly more.

USES OF THE INSTRUMENT—

The instrument has so far been considered as an analytical tool, but it has other important uses. Like other types of polarographs it may be used for certain fundamental physical measurements such as those of diffusion coefficient or the number of electrons involved in a reduction or oxidation process. It is particularly useful for rapidly checking the efficiency of electrolytic separations, and the relatively short time required for an observation (this can be reduced to about 4 secs.) makes it a valuable means for following the course of rapid reactions, *e.g.*, tautomeric conversions. However a particular virtue lies in the fact that the shape of the reduction (or oxidation) peak is indicative of the nature of the reaction. Ions such as those of cadmium, thallium and lead are characterised by the fact that their reductions are thermodynamically reversible. Other ions such as those of stannic tin complexes, nickel in sulphate media, and a large number of reducible organic compounds do not show this ease of reduction but require an overvoltage to effect the change. The result is to introduce a rate-controlling process other than diffusion, and this manifests itself as a rounding and flattening of the polarographic peak. For truly reversible reactions the curves can be treated mathematically in a manner analogous to that used in deriving the Ilkovic equation, but so far little or no work has been done in attempting to treat irreversible reactions in this manner. Apart from the mathematical aspect of the matter, the appearance of the polarograms is a very useful criterion of the suitability of a reaction for analytical or electrochemical work.

It may be useful to consider briefly certain recent developments. First, it has been shown that platinum micro-electrodes may be used in the region +1 to —0·3 volt (versus S.C.E.) with some success. Secondly, it has been shown that the dropping mercury electrode may be replaced by an amalgamated silver or preferably an amalgamated platinum micro-electrode. Providing that amalgamation is good, such electrodes may be used up to —2·1 volts without difficulties due to hydrogen evolution. The technique adopted with these "film electrodes," as they may be described, is to execute a voltage sweep in the ordinary way and to observe the polarogram in a manner similar to that described above. The potential is then returned to a point below the half-wave potential of the ion in question and maintained there for about 10 seconds, during which period the deposited ion is redissolved and concentration gradients are practically eliminated. As will be apparent, it is essential that the "film" be regenerated during the latter part of each cycle and in consequence the electrode can be used only for such metals as will dissolve easily from an amalgam. In the case of reduction (or oxidation) from one valency state to another the limitation does not of course apply.

Although much work remains to be done on this type of electrode it would appear that its main advantage lies in the great simplification in the circuit of the apparatus. The electrodes may be made of constant and accurately reproducible dimensions and appear to be reliable in operation. The use of a synchronising circuit is no longer necessary and hence a much simpler polarogram-measuring technique may be employed. Moreover it becomes possible to enhance the sensitivity of the method by increasing the surface area of the electrode beyond that normally obtainable from a capillary tube. A point of theoretical interest which has not yet been investigated is that with both types of film electrodes some maximum suppressor appears to be necessary when dealing with certain ions.

In conclusion a comparison of the three types of instrument—photographic, pen-recording and cathode ray tube polarographs—under various headings will be given.

(1) *Accuracy*—There are no very significant differences.

(2) *Sensitivity and Robustness*—It is considered that the cathode ray instrument is much superior to the other types owing to the lack of delicate moving parts. However, circuit characteristics would set a practical limit to an increased sensitivity at about one order greater than commercial equipment.

(3) *Adaptability*—As an analytical tool the instrument has the merit of speed for routine work. As with the pen-recorder type, any malformation of the polarogram may be easily detected, but the lack of a permanent record may be a drawback.

(4) *Complexity and Size*—On this score the apparatus is obviously inferior to the other types. Even with improved design it will require approximately twice the space required by a pen-recording instrument and some considerable knowledge of electronics may be required to trace and remedy any circuit faults that may develop.

(5) *Cost*—A quite satisfactory apparatus can be built with limited workshop facilities, and it is thought that the cathode ray polarograph should be no more expensive than the other types.

The authors wish to acknowledge valuable advice and criticism from Mr. S. H. Bales, Principal Scientific Officer and Mr. A. S. Nickelson, Principal Scientific Officer of the Chemical Inspection Department, Ministry of Supply.

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ROYAL ARSENAL, WOOLWICH

DISCUSSION

Mr. A. C. **MASON** asked if the apparatus described was more effective than the ordinary polarograph in separating waves whose half-wave potentials are close together.

Dr. **ZADE** asked if dI/dE curves had been plotted on the cathode ray oscillograph, as in the method used by Heyrovsky recently.

Dr. J. G. A. **GRIFFITHS**, referring to the use of "film electrodes" mentioned by Mr. Airey, asked if it was satisfactory to deposit a film of silver on a metal into which mercury does not diffuse.

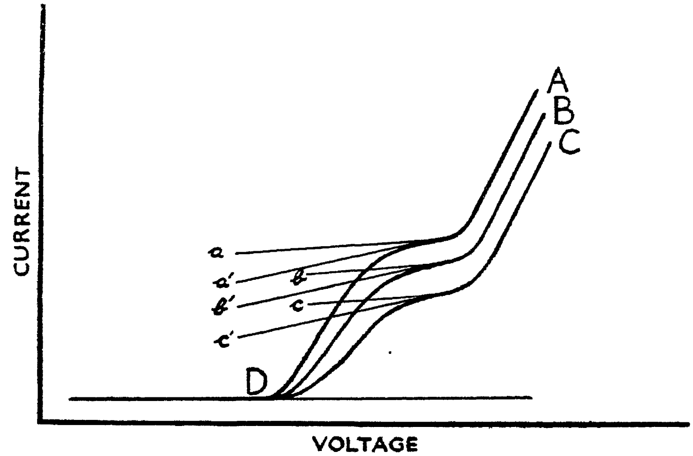
Mr. K. **GOLDSCHMIDT** said that Messrs. Randles and Airey had described a spectacular, but rather complicated instrument, and he was doubtful if it could be operated by an unqualified assistant. Had the authors had experience with simpler circuits in which the cathode ray oscillograph is employed as an indicator in conjunction with the usual polarographic measuring or recording equipment? He had used a modification of a method first described by Boeke and van Suchtelen (*Z. Elektrochem.*, 1939, **45**, 753), in which a small A.C. bias superimposed on the electrode potential permitted accurate detection of half-wave potential and of changes of curvature at the beginning and end of a wave. From the latter the concentration could be derived by noting the difference between the corresponding deflections of a galvanometer, or alternatively by recording the desired part of a wave on a polarograph. Complete analysis of a solution could thus be carried out very rapidly, and in some instances continuously. The cathode-ray oscillograph may be of any commercial type, and the only other components necessary are a few radio-type resistors and capacitors. Interpretation of the pattern obtained on the cathode-ray tube is very simple, and the device can be used by an unqualified investigator.

Mr. **AIREY**, in reply to Mr. Mason, said the instrument was an improvement, but not a great one, on the ordinary polarograph in the separation of waves that were close together. Waves 0·15 volt apart could be separated but that was about the limit. He had not yet attempted the plotting of dI/dE curves with the instrument. In reply to Dr. Griffiths he said that they had obtained satisfactory film electrodes by dipping red-hot platinum into mercury.

Notes

AIDS TO ACCURACY IN QUANTITATIVE ESTIMATION FROM SIMPLE POLAROGRAMS

In quantitative polarographic estimations it is often a matter of some difficulty to decide the exact angle at which to draw the tangent to a diffusion current curve, as, for example, the choice between *a* and *a′* in the accompanying diagram.



In the "internal standard" method of estimation this difficulty can be overcome by making a second addition of a known amount of standard solution. The usual technique according to the "internal standard" procedure is to obtain a "wave" for the unknown, say C, to add a known amount of standard solution of the substance to be estimated, and obtain another "wave" as at B. Then concentration is proportional to wave height, and the latter is found by drawing parallel tangents to B and C and a tangent to the "foot" of the curve (D), the height being measured as the intercept on a vertical drawn at the half-wave potential. It will be obvious that the correct slope of the tangents is a matter of some importance in accurate work.

If now a second addition is made, the ratios of the wave heights for the solutions (making an allowance for the approximate unknown concentration) are fixed, and tangents can be drawn to ensure these ratios. The common slope of all the diffusion current tangents is then completely determinate.

In a case producing a curve similar to that depicted, the intercepts could have been measured as follows:

C = 11 to 14 mm. (unknown concentration)
B = 17 ,, 20 ,, (10 μg. per ml. addition)
A = 24 ,, 27 ,, (20 ,, ,, ,, ,,).

From the average values of C and B, the unknown concentration was about 21·25 μg. per ml. Thus, as a first approximation, the wave-height ratios were

$$\frac{h_A}{h_B} = \frac{41\cdot25}{31\cdot25} = 1\cdot32 \text{ and } \frac{h_B}{h_C} = \frac{31\cdot25}{21\cdot25} = 1\cdot47.$$

The stipulation of both these ratios simultaneously narrows the possible intercept values (as may be seen by setting up the ratios on a slide rule) to:

C = 12·4 to 13·6; B = 18·2 to 20·0; A = 24·0 to 26·4.

Then taking C as 13·0 and drawing parallel tangents to all three curves, there is obtained:

C = 13·0; B = 19·0; A = 26·0

whence the unknown concentration is 20 μg. per ml.

For greater accuracy, successive approximations can be made for the unknown concentration, which process is facilitated by a device for laying on the polarogram, comprising a sheet of transparent material on which a series of parallel lines (*e.g.*, 1 mm. apart, with centimetres marked) is ruled, photographed, or otherwise applied.

The "internal standard" method of estimation is described in "*Polarography*," by Kolthoff, I. M., and Lingane, J. J., Interscience Publishers, New York, 1941, p. 251, and an inspection of Fig. 91 on p. 252 will show the difficulty of measurement in some instances, particularly as the half-wave intercept method mentioned above is now generally used (*cf.*, "*Polarographic and Spectrographic Analysis of High Purity Zinc and Zinc Alloys for Die Casting*," H.M. Stationery Office, 1945, p. 26).

It must be stressed that the method herein described involves a different principle from the known method of making successive additions, measuring wave heights and plotting back to the unknown concentration. At no point in that method is any wave-height accurately fixed, and systematic inaccuracies will be reflected in the final result.

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JOHN A. LEWIS

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CHEMICAL RESEARCH LABORATORY
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ERRATUM: In the issue for June, 1947, p. 253, line 5 from the bottom of the page *for* "County of Essex" *read* "County of Sussex."

Ministry of Food

STATUTORY RULES AND ORDERS*

1947 **876. The Pickles and Sauces Order, 1947. Dated May 7, 1947.** Price 2d.

In this Order, which replaces the Pickles and Sauces Order, 1946 (*S.R. & O.*, 1946, *No.* 2216)—

"Pickles" means sweet pickles (whether mixed with any sauce or not) mixed pickles or piccalilli, or any pickled beetroot, cabbage, capers, cauliflowers, cucumbers, gherkins, mushrooms, olives, onions, pimentos, tomatoes or walnuts, or any other pickled vegetables, fruit or fungi.

"Sauce" means anchovy essence or sauce, caper sauce, chutney, fruit sauce of the type commonly used with meat or fish, mushroom ketchup, horseradish cream or sauce, mayonnaise, mint jelly or sauce, mushroom ketchup or sauce, mustard sauce, prepared mustard, salad cream or any other salad dressing, sandwich spread, tomato ketchup or sauce, walnut ketchup, Worcester sauce, or any other similar products.

No person shall sell any product under the description "tomato ketchup" or "tomato catsup" unless it contains no fruit or vegetable other than tomatoes (except onions, garlic and spices added for flavouring purposes).

— **968. The Edible Gelatin (Control) (Amendment) Order, 1947. Dated May 16, 1947.** Price 1d.

This amending Order

(*a*) *provides that edible gelatin* (*see S.R. & O.*, 1947, *No.* 161; ANALYST, 1947, **72**, 66) *may be manufactured by the blending together of two or more gelatins* (*not necessarily individually complying with the definition of "edible gelatin"*)*; and*

(*b*) *adds jellied eels to the list of foods in the manufacture of which edible gelatin may be used.*

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* Obtainable from H.M. Stationery Office. Italics signify changed wording.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Polarographic Analysis of Streptomycin. G. B. Levy, P. Schwed, and J. Warren (*J. Amer. Chem. Soc.*, 1946, **68**, 528–529)—Under certain conditions, streptomycin gives rise to a well-defined polarographic reduction wave at high *p*H values. At 13·6° C., with 3 per cent. tetramethyl ammonium hydroxide as supporting electrolyte, experimental points over a range of 100 to 1000 units per ml. fall on a straight line with an average deviation of ±13 units. The half-wave potential is approximately −1·45 v. *versus* the mercury anode. The reaction probably involves one electron, and the diffusion coefficient is 3×10^{-6}cm.^{2} sec.$^{-1}$ The temperature coefficient of the diffusion coefficient is 3 per cent. per degree at room temperature, but erratic results are obtained at elevated temperatures. All oxygen must be removed by passing nitrogen through the solution before a polarogram is taken, and if any non-aqueous solvent is present a special calibration curve must be used.

Some streptomycin solutions contain materials that interfere with the analysis. These impurities can be precipitated by lead ions, the excess of lead being removed by precipitation with sulphuric acid.

Results obtained by the polarographic method agree closely with those obtained by microbiological assay. J. G. W.

Biochemical

Estimation of Threonine and Serine in Proteins. M. W. Rees (*Biochem. J.*, 1946, **40**, 632–640)—The method is a modification of that of Nicolet and Shinn (*J. Amer. Chem. Soc.*, 1939, **61**, 1615; 1941, **63**, 1486; *J. Biol. Chem.*, 1941, **138**, 91; 1941, **139**, 687; 1942, **142**, 139), essentially the same method being used and the same apparatus (*cf.* Abst., ANALYST, 1941, **56**, 343).

Procedure—Put about 3·5 and 2·5 ml. of sodium bisulphite solution (5 ml. of a commercial solution, sp.gr. 1·34, are diluted to 100 ml.) into the first and second receiver tubes of the apparatus and dilute to 25 ml. Pipette a sample of the protein or test solution, containing 1·5 to 7 mg. of threonine and 2 to 7 mg. of serine, into a reaction tube and neutralise to *p*H 7 with *N* sodium hydroxide. Add 1·5 ml. of potassium arsenite solution (dissolve 25 g. in water, dilute to 100 ml. and add *N* sulphuric acid until, on addition of 1 ml. of 0·5 *M* periodic acid to 1·5 ml. of the solution in 20 ml. of water, the *p*H is 7·0) followed by 6 ml. of a phosphate buffer solution of *p*H 7·16 (mix 30 ml. of a KH$_2$PO$_4$ solution containing 18·156 g. per litre with 70 ml. of Na$_2$HPO$_4$ solution containing 19·536 g. per litre), adjust the volume to approximately 18 ml. and then, when each of the sets of tubes has been prepared in this way, close the stop-cocks of the separating funnels, and into each funnel put 1 ml. of 0·5 *M* periodic acid and 3 ml. of water. Connect up the apparatus, draw air through it slowly, and open the stop-cocks. After 3 to 4 min., increase the rate of aeration to 1 litre per minute and continue for 1 hour. Stop the flow of air and wash the contents of each pair of receiving tubes into a titration flask. Stopper the flasks and set aside until the formaldehyde distillations are completed.

To estimate the acetaldehyde, oxidise the excess of bisulphite with approximately 0·1 *N* iodine and finally with 0·01 *N* iodine, using 2 ml. of a starch solution as indicator. Add about 7 ml. of *M* sodium bicarbonate and titrate the liberated bisulphite with 0·01 *N* iodine until the fading rate of the blue colour slows down. Add another 2 ml. of the bicarbonate solution, continue the titration, and then add a further 2 ml. and complete the titration; the blue colour should persist for one minute or longer.

After removal of the acetaldehyde by aeration, transfer the contents of each reaction tube quantitatively without undue delay to a micro-Kjeldahl flask into which 0·6 ml. of *N* sulphuric acid has been put. Steam-distil the contents of the flask at such a rate that the volume is about 5 ml. after 30 min. and the volume of distillate is about 250 ml. Titrate the formaldehyde in this distillate in the same way as the acetaldehyde, but use 4 ml. of starch solution, and 5 per cent. sodium carbonate solution in place of the bicarbonate solution. The recoveries of added threonine and serine were 99 ± 1 per cent. and 100 ± 2 per cent., respectively. Satisfactory agreement was obtained with the amounts of ammonia simultaneously produced.

F. A. R.

Precision Method for the Quantitative Determination of Calcium in Blood Plasma. K. Salomon, B. W. Gabrio, and G. F. Smith (*Arch. Biochem.*, 1946, **11**, 433–443)—The method involves preliminary ashing of the plasma with a perchloric acid - nitric acid mixture, precipitation of the calcium as oxalate, and titration of the oxalate ion with ammonium hexanitratocerate in perchloric acid solution, nitro-*o*-phenanthroline ferrous sulphate (nitroferroin) being used as indicator.

Reagents—Ashing mixture, a 1+1 mixture of 72 per cent. perchloric acid (sp.gr. 1·69) and concentrated nitric acid (sp.gr. 1·42, analytical reagent). Triton, N.E. (1:30 dilution of original solution from Rohm and Haas Co., Washington Square, Philadelphia). Two per cent. aqueous ammonia, prepared daily (distil concentrated aqueous ammonia and prepare a 10 per cent. stock solution from which the 2 per cent. solution is obtained). Saturated solution of ammonium oxalate analytical reagent, kept at room temperature. 0·001 *N* Sodium oxalate, primary standard, analytical reagent (the salt is dissolved in 2 *N* perchloric acid; in the absence of perchloric acid, the solution is not stable on storage at this concentration). 0·00025 *N* Nitro-*o*-phenanthroline ferrous sulphate indicator (obtained from G. Frederick Smith Chemical Co., Columbus, Ohio, in 0·025 *M* solution, to be diluted with re-distilled water). Ammonium hexanitratocerate, standard or reference purity (G. Frederick Smith Chemical Co.). Prepare a 0·01 *N* solution of the cerium salt in 2 *N* perchloric acid by adding slowly 167 ml. of 72 per cent. perchloric acid to 5·4819 g. of ammonium hexanitratocerate, stirring constantly; then add slowly, with constant stirring, re-distilled water to 1000 ml.; slow addition of 50 ml. of water with stirring for 1 min. after each addition is essential. Dilute the resulting 0·01 *N* perchloratoceric acid with 2 *N* perchloric acid to 0·001 *N* concentration, and standardise with 0·001 *N* sodium oxalate solution. Store the solution in the dark and in a refrigerator to increase its stability with age.

Procedure. Ashing—Pipette 0·5 ml. of plasma or serum into a 25-ml. Erlenmeyer flask and add 2 ml. of the ashing mixture. Heat on a hot plate, beginning the ashing at 180° C.; if a plasma of high lipid content is being ashed, the initial temperature should be 100° C. to avoid spattering. Raise the temperature slowly to 240° C.; the temperature should never exceed 250° C. Ashing is complete when there are no remaining drops of liquid and no more white fumes escaping, and when only the snow-white, dry ash remains in the flask. It is important that no traces of perchloric acid be left in the flask as the perchlorate ion interferes with quantitative precipitation of the amounts of calcium oxalate concerned in this procedure. The ashing takes about 3 to 4 hours, which may be shortened by brushing the top half of the flask with a small, colourless gas flame at the end of the digestion to minimise refluxing of the acid condensate on the inside walls of the flask, and to facilitate elimination of the excess of acid.

Precipitation of the calcium as oxalate—Dissolve the ash while it is still hot with 2 ml. of 0·04 *N* hydrochloric acid, using gentle heating over a micro-flame if necessary. Transfer the dissolved ash with re-distilled water (total about 5 ml.) to a conical, 15-ml. centrifuge tube. Add 2 ml. of saturated ammonium oxalate solution and leave for 16 hours at room temperature for complete precipitation. The *p*H is always 4·0 to 6·0, which is within the optimum range for the precipitation of calcium oxalate as indicated by Sendroy (*J. Biol. Chem.*, 1944, **152**, 539).

Treatment of the precipitated calcium oxalate—The procedure of Sendroy (*loc. cit.*) is followed, with slight modifications. To the solution containing the calcium oxalate precipitate add 0·2 ml. of Triton before centrifuging at 2600 r.p.m. for 5 min. Slowly siphon off the supernatant liquid with a hooked capillary, allowing about 0·2 ml. of the liquid to remain in the tube. Wash the precipitate with 3 ml. of 2 per cent. aqueous ammonia, allowing 1 ml. of this solution to wash down the sides of the tube slowly and adding the remaining 2 ml. more rapidly, also down the sides of the tube. Centrifuge again at 2600 r.p.m. for 5 min. Draw off the supernatant liquid as before and wash for a second time in the same way. After the third centrifugation and withdrawal of the fluid, dissolve the precipitate immediately in 4 ml. of 2 *N* perchloric acid, with stirring. Transfer the solution to a 25-ml. beaker and wash the centrifuge tube twice with a total volume of 6 ml. of 2 *N* perchloric acid.

Titration—Immediately before titration, add 0·5 ml. of 0·00025 *N* nitroferroin indicator to produce a distinct pink colour. Titrate with 0·001 *N* perchloratoceric acid to a sharp colour change from pink to colourless, using a 10-ml. micro-burette (Koch) graduated in 0·05 ml., and having a reservoir and a standard capillary tip. One ml. of 0·001 *N* perchloratoceric acid is equivalent to 20 µg. of calcium.

Standardisation of perchloratoceric acid solution—Standardise the 0·001 *N* perchloratoceric acid in 2 *N* perchloric acid in the same manner as the titration of the oxalate from calcium oxalate, using a 0·001 *N* solution of sodium oxalate in 2 *N* perchloric acid. For 10 ml., use 0·5 ml. of 0·00025 *N* nitroferroin indicator. The blank correction for the indicator thus amounts to approximately 0·1 ml. of oxidant; this correction can be duplicated precisely, and therefore introduces no error. A 0·001 *N* perchloratoceric acid solution in perchloric acid is stable for about 4 days in the dark at about 0° C.

Modification of procedure using 3 *ml. of plasma*—Pipette 3 ml. of plasma or serum into a 25-ml. vitreosil crucible and dry at about 100° C. Ash over a Bunsen burner until nearly all the material is white, and then complete the ashing by adding 2 ml. of ashing mixture and heating on a hot plate at about 240° C. until a dry, white ash is obtained; the total time required for ashing is about 2 hours. Dissolve the ash in 5 ml. of 0·04 *N* hydrochloric acid, transfer the solution with a total of 15 ml. of water to a 40-ml. centrifuge tube, add 4 ml. of saturated ammonium oxalate solution, and leave for 16 hours. No *p*H adjustment is necessary. Treat the precipitated calcium oxalate as above, but use 4 ml. of 2 per cent. aqueous ammonia for each washing. For the final titration, use 0·01 *N* perchloratoceric acid with the same quantity and concentration of nitroferroin indicator as above.

Evidence which suggests that the presence of increased amounts of lipids interferes with direct precipitation methods of determining calcium is presented, and the above procedure involving preliminary ashing is recommended. E. M. P.

Determination of Serum Phosphate by the Molybdi-vanadate Method. D. G. Simonsen, M. Wertman, L. M. Westover, and J. W. Mehl (*J. Biol. Chem.*, 1946, **166**, 747–755)—The molybdivanadate method for orthophosphate described by Kitson and Mellon (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 379) is suitable for the estimation of serum phosphate.

Procedure—Add 2 ml. of serum to 6 ml. of 10 per cent. trichloroacetic acid in a 15-ml. centrifuge tube. Mix by inversion, leave for 15 min., and then centrifuge for 7 to 10 min. at 2500 to 3500 r.p.m., or until the supernatant liquid is clear. Transfer 3 ml. of the supernatant liquid to a Klett tube graduated at 5 ml. Add 0·5 ml. of diluted nitric acid (1+2), followed by 0·5 ml. of 0·25 per cent. ammonium vanadate solution (prepared by dissolving 2·5 g. of the salt in about 500 ml. of boiling water, cooling slightly, adding 20 ml. of concentrated nitric acid, allowing to cool to room temperature, and then diluting to 1 litre). Next add 0·5 ml. of 5 per cent. ammonium molybdate solution and dilute to 5 ml. with water. Leave for 5 min. and evaluate the colour in a Klett colorimeter with a No. 42 (blue) filter. Prepare a blank with 3 ml. of 7·5 per cent. trichloroacetic acid, treat this in the same way as the test solution, and subtract the reading thus obtained from the reading of the test solution. A spectrophotometer may be used for evaluating the colour. Calculate the phosphorus content from a standard curve obtained by treating a standard solution of potassium dihydrogen phosphate (0·4389 g. in 1 litre of 7·5 per cent. trichloroacetic acid) in the same way. The standard solution contains 100 μg. of phosphorus per ml., and to prepare the curve, solutions giving a range of 5 to 80 μg. of phosphorus in the final 5 ml. are used.

Recoveries of phosphate added to serum ranged from 99·3 to 101·0 per cent. of the theoretical.

F. A. R.

Yeast Microbiological Method for Estimation of Nicotinic Acid. W. L. Williams (*J. Biol. Chem.*, 1946, **166**, 397–406)—The method utilises a yeast, *Torula cremoris*, the growth of which is estimated turbidimetrically. The method is rapid, 16 to 18 hours being allowed for growth, and specific. It can be used for the differential assay of nicotinic acid, trigonelline, and N^1-methylnicotinamide.

Procedure—Maintain stock cultures on Difco malt agar for 24 hours at 37° C. for a period not exceeding one month. Make a fresh transfer 24 hours before each series of assays and incubate at 37° C. Transfer the yeast from this culture to 10 ml. of sterile saline to give a suspension with 15 to 25 per cent. absorption in an Evelyn colorimeter. Add the contents of the tube to 90 ml. of sterile saline and use the suspension for inoculating the assay tubes at the rate of 1 ml. per tube. Prepare a basal medium of the following composition

Component	Amount per 10 ml. of final medium
	mg.
Glucose, anhydrous	500
KH_2PO_4	3
$MgSO_4$	1
	ml.
Charcoal-treated peptone	1(=100 mg.)
Potassium citrate buffer (1 litre contains 100 g. of potassium citrate and 20 g. of citric acid)	0·5
	μg.
Biotin	0·25
Aneurine hydrochloride	25
Pyridoxine hydrochloride	25
Calcium pantothenate	25
Inositol	250

Weigh out an amount of sample estimated to contain 5 to 10 μg. of nicotinic acid, suspend in a minimum quantity of water, add 50 ml. of 3 *N* sodium hydroxide, and autoclave at 15 lb. pressure for 1 hour. Cool, adjust to *p*H 5·0 to 5·5 with 3 *N* sulphuric acid and dilute to 200 ml. Centrifuge, if necessary, to obtain a clear extract or, if starch is present, incubate at 100° C. for 5 min. with 2 to 4 mg. of taka-diastase and then centrifuge. Milk and milk products may become highly coloured by the alkaline treatment and, as these do not contain trigonelline or N^1-methylnicotinamide, *N* sulphuric acid may be substituted for the 3 *N* sodium hydroxide.

Put 5 ml. of the double-strength basal medium into a series of standard Evelyn test tubes and to 8 of these add 0, 0·5, 1·0, 1·5, 2·0, 2·5, 3·0, and 4·0 ml. of a standard solution of nicotinic acid containing 50 millimicrograms per ml. Into another 4 test tubes put 1·0, 2·0, 3·0, and 4·0 ml. of the solution to be tested, and adjust the volume in each test tube to 9 ml. by adding water. Plug each with cotton wool, steam for 10 min., cool, and then add 1 ml. of inoculum. Shake the tubes in an incubator at any temperature between 30 and 38° C., and measure the turbidity after 16 to 18 hours. Calculate the nicotinic acid content of the test solution from the standard curve. The recovery of nicotinic acid added to various materials was 95 to 99 per cent. of the theoretical.

Differential assays of nicotinic acid and its two derivatives can be carried out by treating the sample with: (*a*) 50 ml. of water, (*b*) 50 ml. of 2 *N* sulphuric acid, and (*c*) 50 ml. of 3 *N* sodium hydroxide, autoclaving at 15 lb. for 1 hour, neutralising, diluting, and assaying each solution as described above. The response induced by the first solution is due to nicotinic acid and trigonelline, by the second solution to all 3 compounds, and by the third solution to nicotinic acid alone.

F. A. R.

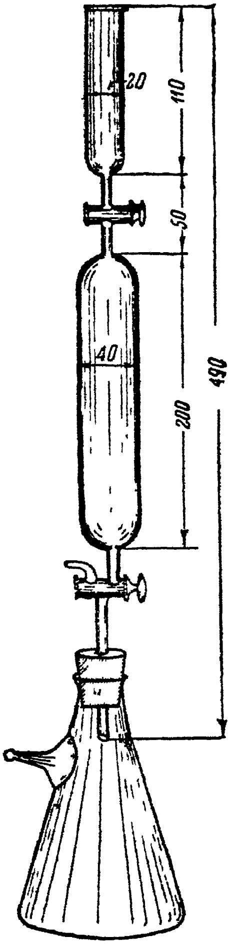
Chemical Assay Method for Penicillin G. J. C. Sheehan, W. J. Mader, and D. J. Cram (*J. Amer. Chem. Soc.*, 1946, **68**, 2407)—The method depends on the low solubility of the N-ethylpiperidine salt of penicillin G in amyl acetate - acetone mixtures. Penicillins K and F, and the degradation products of G are said not to interfere. The method is claimed to be quantitative with penicillin G contents exceeding 50 per cent., and with potencies exceeding 800 units per mg.

Procedure—Transfer, by means of a 2-ml. syringe inserted through the rubber cap, the contents of a weighed penicillin vial (100,000 or 200,000 units) to a chilled centrifuge tube, using 3 ml. of ice-cold water. Dry the bottle and weigh. Add to the aqueous solution exactly 2 ml. of ice-cold amyl acetate saturated with N-ethylpiperidine salt of penicillin G (the solubility is approximately 0·6 mg. per ml.). Add, with shaking and cooling in ice, 0·5 ml. of 20 per cent. phosphoric acid solution and centrifuge. Remove about 1·8 ml. of the amyl acetate layer, and dry with 0·1 g. of sodium sulphate. Filter through a sintered-glass, micro-filter-crucible. The *p*H of the spent aqueous layer should be about 2. Transfer exactly 1 ml. of the dried amyl acetate solution to a 10-ml. micro-beaker in an ice-bath. Add 1 ml. of acetone saturated with the N-ethylpiperidine salt of penicillin G (the solubility is approximately 2 mg. per ml.) followed by 0·5 ml. of a 10 per cent. solution of N-ethylpiperidine in amyl acetate saturated with the amine salt (about 2 mg. per ml.). After 2 hours at 0° to 5° C., filter through a weighed micro-filter stick, wash with 1 ml. of cold acetone (saturated with amine salt), and dry *in vacuo* at room temperature for 1 hour. The practically colourless N-ethylpiperidine salt of penicillin G melts, with decomposition, at 152 to 154° C., when placed in a capillary in a bath at 140° and heated at a rate of 3° per min.

W. S. W.

Organic

Determination of Low Concentrations of Ethyl Alcohol in Aqueous Solutions. N. V. Tschalov and L. P. Volskaja (*Zavod. Lab.*, 1946, **12**, 286–291)—The determination of low concentrations (0·02 to 1 per cent.) of alcohol in aqueous solutions containing large proportions (5 to 200 times that of the alcohol) of aldehydes, ketones, esters, furfural, etc. (*e.g.*, industrial solutions obtained in the preparation of alcohol from sulphited pulp) is described. The method is based on the formation of ethyl nitrite, which is highly volatile (b.p. 18° C.) (Fischer and Schmidt, *Ber.*, 1924, **57**, 693, and 1926, **58**, 679; Skrabal, *Z. anal. Chem.*, 1940, **119**, 222). All variations of this method that have been described previously depend on the removal of the nitrite from the reaction vessel by means of carbon dioxide, nitrogen, or air, and its subsequent absorption in a solvent (*e.g.*, carbon tetrachloride); the necessary apparatus is complicated, the time required is long, and the purification of the solvents is tedious. Such a method is, therefore, unsuitable under works conditions.



The modification now described consists in the retention of the nitrite in the gaseous form in the reaction vessel and the removal of the reaction liquid; the ethyl nitrite is then saponified in the reaction vessel, the nitrous acid is allowed to react with sulphanilic acid, and the diazo compound is coupled with α-naphthylamine to give an orange azo dye which can be estimated colorimetrically.

The reaction vessel is a glass cylinder, capacity 200 ± 5 ml., joined through a tap at the top with a funnel used for delivery of the reagents, and through a two-way vacuum tap at the bottom to a glass tube which passes down into a pressure filtering flask through a rubber bung (see Figure; the dimensions given are in mm.).

Procedure—By means of a water-pump or other means reduce the pressure to about 500 mm. of mercury in the reaction vessel, and then introduce successively through the upper tap 1 ml. of 50 per cent. sodium nitrite solution, 20 ml. of the solution under examination, previously diluted to a concentration of 0·025 to 0·005 per cent. of alcohol, and 1 ml. of 20 per cent. hydrochloric acid, without admitting any air. Shake for 30 sec. Ethyl nitrite is formed and enters the space above the liquid, but oxides of nitrogen are also present and they must be removed by adding 2 ml. of 25 per cent. sodium hydroxide solution and shaking vigorously five to eight times. Connect the flask to a pump and when the pressure is less than that in the reaction vessel open the lower tap and allow all but a small quantity of liquid (3 to 5 mm. height in the tube above the tap) to flow into the flask. Purify the ethyl nitrite further by introducing simultaneously 0·5 ml. of 25 per cent. sodium hydroxide solution and 10 ml. of 2 per cent. potassium permanganate solution and shaking for 30 sec. Remove the bulk of the solution, and wash out the vessel twice with 10 ml. of distilled water. Now add to the purified ethyl nitrite 10 ml. of sulphanilic acid solution (6 g. of sulphanilic acid in one litre of 0·1 *N* hydrochloric acid), shake for 30 sec., run in 10 ml. of α-naphthylamine solution (3·2 g. of the amine in 1 litre of water containing 6 ml. of 20 per cent. hydrochloric acid), allow to stand for 2 min., add 10 ml. of 15 per cent. sodium hydroxide solution, make up to 250 ml. with distilled water, and determine the colour intensity by means of a photometer with potassium photo-electric cell. Evaluate the alcohol content of the original solution from calibration curves obtained from the use of standard solutions.

The total time for a determination is 20 min., and an accuracy of 3 parts in 100 is attainable, but the conditions as given above are fairly critical. The formation of ethyl nitrite is not quite quantitative and some loss occurs later; hence the results must be referred to those obtained with standards under the same conditions. The indicated size of the reaction vessel and volumes of solutions must be observed; some extension of the times of shaking and colour development is permissible. Liquors from hydrolysis works can be analysed directly, but those from sulphite works should be first distilled to avoid the formation of foam in the reaction vessel. Alcohols other than ethyl alcohol react similarly, but volatile products are not formed from the other components of the solutions. G. S. S.

Determination of Linoleic Acid in Cholesteryl Linoleate. J. S. Front and B. F. Daubert (*J. Amer. Chem. Soc.*, 1945, **67**, 1509–1510)—If cholesteryl linoleate could be directly isomerised in alkaline ethylene glycol solution and the spectral absorption of the isomerised product measured by the method of Mitchell, Kraybill, and Zscheile (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 1; *cf.* ANALYST, 1942, **67**, 310), a satisfactory method of ascertaining its purity would result. It was found that, unlike most fats, synthetic glycerides and potassium linoleate, cholesteryl linoleate cannot be isomerised directly in alkaline ethylene glycol or glycerol by the method cited. When heated with the alkaline glycol at 180° C. for 30 min. in an oil bath, it melts and rises to the surface. The average $E^{1\%}_{1\,\text{cm.}}$ value at 234 mμ. of the product, *viz.*, 46, indicates that although partial hydrolysis has occurred it represents only 12·3 per cent. of the theoretical value of 374 based on an experimentally determined $E^{1\%}_{1\,\text{cm.}}$ value of 867 for linoleic acid.

The alkaline glycol used in the method was prepared by dissolving 7·5 g. of potassium hydroxide in 100 ml. of redistilled ethylene glycol. The solution was heated before use to 190° C., cooled to room temperature, and made up to 100 ml. with the glycol. About 0·1 g. of cholesteryl linoleate was weighed in a 1″ × 6″ Pyrex test tube. One ml. of alcoholic potassium hydroxide (55 g. to 100 ml. of 99 per cent. alcohol) was added to the sample and to a blank tube. The tubes were warmed in a water-bath at 55° to 60° C. for 90 min., after which 10 ml. of alkaline ethylene glycol were added to both sample and blank, and the tubes were placed in a constant temperature bath at 180° C. for 30 min. in an atmosphere of nitrogen. At three successive 1-min. intervals the tubes were removed from the bath and shaken thoroughly for proper mixture of ester and reagent. The slight frothing that occurred during the heating process did not interfere with isomerisation. After exactly 30 min. of total heating time the tubes were removed from the bath and immediately cooled in water. The isomerised soaps and excess of reagent were transferred with 99 per cent. alcohol to volumetric flasks and further diluted until their optical densities were suitable for measurement in the spectrophotometer.

The $E^{1\%}_{1\,\text{cm.}}$ value of the linoleic acid from which the ester was prepared was 867. Comparison of the calculated theoretical $E^{1\%}_{1\,\text{cm.}}$ value of 374 for cholesteryl linoleate with the average value found experimentally, *viz.*, 373 indicated a purity of the ester of 99·7 per cent.

Absorption measurements made with a Beckman spectrophotometer of a solution of cholesteryl linoleate in *iso*-octane solution showed a total diene conjugation of less than 0·1 per cent. and the triene conjugation was negligible. Corrections for preformed conjugation were, therefore, not made in the final $E^{1\%}_{1\,\text{cm.}}$ values.

The ester used in the investigation was prepared from cholesterol and linoleyl chloride by the method of Page and Rudy (*Biochem. Z.*, 1930, **220**, 304) with the following modifications. After reaction, the cooled liquid ester was dissolved in 300 ml. of ethyl ether and the solution was washed with a 5 per cent. potassium carbonate solution and with water. The ether was removed from the dried and filtered liquid under nitrogen, and the liquid residue was dissolved in a small volume of ether. Sufficient ethyl alcohol was then added to impart a slight turbidity to the solution and crystallisation of the ester occurred on cooling overnight at 0° to 5° C. Recrystallisation several times from the same mixture of solvents yielded a product melting sharply at 42·5° C. The ester was also prepared by the following method. Cholesterol (5 g.) dissolved in a mixture of 20 ml. of chloroform and 5 g. of quinoline was refluxed on a steam-bath for 3 hr. with 3·9 g. of linoleyl chloride, and, when cold, the product was dissolved in 300 ml. of ethyl ether. The solution was washed successively in a separating funnel with 30-ml. portions of 0·5 *N* sulphuric acid, 5 per cent. potassium carbonate solution, and water. After being dried over anhydrous sodium sulphate the solution was treated in the manner already described. The m.p. of the crystallised product was 42° to 42·5° C.

The purity of cholesteryl linoleate deduced from its linoleic acid content as determined by this method is more reliable than that based on the iodine value. The method should be equally applicable to the determination of the purity of cholesteryl linolenate. A. O. J.

Iodimetric Micro-titration for Mustard Gas. V. E. Kinsey and W. M. Grant (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 794–797)—The method is based on the reaction of dichloramine-T with mustard gas. The residual chlorine in the dichloramine, after reaction with the mustard gas, is determined by titration of the iodine liberated from potassium iodide in presence of acetic acid, the end-point being sufficiently clear to render the use of starch indicator unnecessary. Under the conditions specified, about 10 equivalents of chlorine are used per molecule of mustard gas.

The stability of dichloramine-T was found to be satisfactory in purified kerosene, technical carbon tetrachloride, *cyclo*hexane, benzene, and chloroform, but there was appreciable loss of chlorinating power in xylene and in light petroleum. Accordingly, the dichloramine-T was dissolved (0·2 per cent.) in carbon tetrachloride and the mustard gas, in concentrations of 50 and 150 μg. per ml., in purified kerosene or in *cyclo*hexane. One ml. of the chlorinating solution was then added to 1 ml. of the mustard gas solution and samples for titration were withdrawn periodically during 30 min. The reaction was found to be essentially complete in 20 min. and this reaction period was adopted in all subsequent experiments. Since the amount of mustard gas is represented by a difference in the amounts of sodium thiosulphate required to titrate a sample and a blank determination, it is desirable to keep the amount of dichloramine-T present at a minimum value. As the degree of chlorination of the mustard gas increases slightly over the concentration range of 0·1 to 0·2 per cent. of dichloramine-T, 0·2 per cent. was chosen as the standard concentration. The extent of the reaction was found to be dependent upon the nature of the solvents employed. Carbon tetrachloride was ultimately selected as the best solvent for the dichloramine-T, and the mustard gas was dissolved in either *cyclo*hexane or in purified kerosene. Results vary somewhat with different lots of *cyclo*hexane and, consequently, standard curves should be constructed for each new supply of solvent unless C.P. grade *cyclo*hexane is available. The latter gives a reproducible equivalent of 0·67 ml. of 0·01 *M* sodium thiosulphate per 100 μg. of mustard gas. During a search for the impurity in "practical" (Eastman Kodak) *cyclo*hexane responsible for the variation mentioned, it was found that the presence of *cyclo*hexanol greatly increased the sensitivity of the test, but, although the sensitivity could be increased twenty-fold, the effect was evident only over an extremely narrow concentration range of *cyclo*hexanol in pure *cyclo*hexane or purified kerosene. Advantage is taken of the sensitising action of *cyclo*hexanol in Procedure II (*infra.*).

Procedure I—Allow 1 ml. of mustard gas solution to react at 27° C. with 1 ml. of a 0·2 per cent. solution of dichloramine-T in carbon tetrachloride for 20 min. Add 4 drops of saturated potassium iodide solution and 4 drops of glacial acetic acid, shake the mixture, and add 0·01 *M* sodium thiosulphate until the colour of iodine no longer persists. Vigorous shaking is necessary, as the end-point is approached, and the whole procedure should be carried out away from direct sunlight, best results being obtained by maintaining uniform temperature conditions.

Interference of derivatives of mustard gas was negligible, especially when the solution of the sample in the organic solvent was first extracted with water. Cornea and rabbit blood (representing biological material) exerted no interfering action provided that purified kerosene, xylene, or *cyclo*hexane was used for the extraction. When the other layer is water, most of the mustard gas (98 to 99 per cent.) remains in the purified kerosene or *cyclo*hexane; when the other layer is blood, *cyclo*hexane retains most of the mustard gas (97 per cent.), but kerosene retains only 88 per cent.

Procedure II—This procedure provides the greatest sensitivity in the range 0·2 to 5 μg. of mustard gas. To 1 ml. of a 5 per cent. by volume solution of *cyclo*hexanol in pure *cyclo*hexane or in purified kerosene, add 1 ml. of *cyclo*hexane containing up to 5 μg. of mustard gas, and place the mixture in a test tube in a water-bath thermostatically controlled to ±0·1° C. in the range 25° to 28° C. Add 1 ml. of a 0·1 per cent. dichloramine-T solution in carbon tetrachloride and, exactly 20 min. later, add 4 drops of saturated potassium iodide solution and 4 drops of glacial acetic acid. Titrate the liberated iodine with 0·01 *M* sodium thiosulphate to a colourless end-point. A. O. J.

Chromatography of Sugars and their Derivatives. L. W. Georges, R. S. Bower, and M. L. Wolfrom (*J. Amer. Chem. Soc.*, 1946, **68**, 2169–2171)—A synthetic hydrated calcium acid silicate, known as Silene EF, has been found suitable for the chromatographic separation of sugars and their derivatives. To develop the columns, dioxan and alcohols of low molecular weight were used, after addition of varying amounts of water. The columns are extruded from the tubes, and the positions of the zones are ascertained by "streaking" with

TABLE I

GROUP 1—Sugars, sugar alcohols, and glycosides: (0·5 ml. of 90 per cent. dioxan followed immediately by a solution of 2 mg. of the substance in 0·2 ml. of 90 per cent. dioxan).

Class 1—(10 ml. of 90 per cent. dioxan.)
α-*d*-Galacturonic acid
Lactose monohydrate
Lactitol
Dulcitol
Melezitose, raffinose pentahydrate, gentiobiose, *d*-gluco-*d*-gulo-heptose
Sucrose, maltose monohydrate, cellobiose, *d*-glucitol (sorbitol)
d-Galactose, *d*-mannitol
d-Glucose, *d*-fructose, *d*-mannose, *l*-sorbose
l-Fucitol
l-Arabinose

Class 2—(5 ml. of 90 per cent. dioxan.)
l-Fucose
d-Xylose, *l*-rhamnose monohydrate
Methyl α-*d*-glucopyranoside

GROUP II—Acetylated sugars and acetylated sugar alcohols: (0·5 ml. of benzene followed immediately by a solution of 2 mg. of the substance in 0·5 ml. of benzene)

Class 1—(15 ml. of 250:1 v/v benzene - absolute ethanol)
Raffinose hendeca-acetate
β-Melibiose octa-acetate, sucrose octa-acetate
β-Maltopyranose octa-acetate

Class 2—(15 ml. of 500:1 v/v benzene - absolute ethanol)
Keto-*d*-fructose penta-acetate
d-Glucitol (sorbitol) hexa-acetate, *d*-mannitol hexa-acetate
β-*d*-Glucopyranose penta-acetate
α-*d*-Arabinopyranose tetra-acetate

Class 3—(15 ml. of 1000:1 v/v benzene - absolute ethanol)
α-*l*-Fucose tetra-acetate

GROUP III—Methylated sugars: (0·5 ml. of benzene followed immediately by a solution of 2 mg. of the substance in 0·5 ml. of absolute chloroform)

Class 1—(15 ml. of 100:1 v/v benzene - absolute ethanol)
2:3-Dimethyl-*d*-glucose
2:3:6-Trimethyl-*d*-glucose

Class 2—(12·5 ml. of 250:1 v/v benzene - absolute ethanol)
2:3:4:6-Tetramethyl-*d*-glucose

TABLE II

Substance	Adsorbate soln.	Size of column, cm.	Developer	Distance of zones from top, cm.
100 mg. *d*-Glucitol	30 ml.	5·1 × 24·0	1800 ml.	5 –10·5
100 ,, *d*-Mannitol	90% dioxan		92% dioxan	15·0–21·5
800 ,, Raffinose, $5H_2O$	33 ml.	5·1 × 25·5	500 ml.	1·5– 4·7
800 ,, Sucrose	90% abs. ethanol		95% propanol-2	11·2–21·0
500 ,, *d*-Galactose	20 ml.	4·4 × 19·5	218 ml.	2·3– 4·4
500 ,, *l*-Rhamnose, H_2O	90% dioxan		92% dioxan	13·7–18·0
500 ,, Maltose, H_2O	20 ml.	4·4 × 17·0	300 ml.	1·0– 3·3
500 ,, *d*-Glucose	85% dioxan		92% dioxan	4·5– 6·8
250 ,, *d*-Glucitol hexa-acetate ..	33 ml.	5·1 × 26·0	1800 ml.	9·0–14·8
250 ,, *d*-Mannitol hexa-acetate ..	Benzene		benzene - abs. ethanol (1000:1)	16·5–20·0

alkaline potassium permanganate solution. With this adsorbent, sugars can be chromatographed directly without the necessity of preparing derivatives. Table I gives the chromatographic adsorption series of some sugars and their derivatives arranged in decreasing order of adsorptive strength. Columns, 11 cms. long and 0·9 cm. in diameter, of a 5:1 mixture of Silene EF and Celite were used. The nature of the adsorbate solution is recorded under the Group headings and that of the developer solution under the Class headings.

The reagent used for "streaking" the column was a 1 per cent. solution of potassium permanganate in 2·5 *N* sodium hydroxide; this formed brown zones on reaction with the adsorbed sugars. In Table I, substances on the same line are not separated from one another; separation can sometimes be effected by using different developers as shown in Table II. W. S. W.

Inorganic

Determination of Boric Oxide in Glass. M. Hollander and W. Rieman (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 788–789)—The method gives results with a mean error of 0·04 per cent. of boric oxide with glasses containing less than 15 per cent. *Procedure*—(*A*) *Using a p*H *meter*—Fuse 0·5 g. of glass with 3 g. of sodium carbonate for 10 min. Dissolve the melt in 20 ml. of 6 *N* hydrochloric acid and add 6 *N* sodium hydroxide until the *p*H is between 5·0 and 5·5. Aluminium, iron, and similar elements and most of the silica are precipitated. Sweep out carbon dioxide by bubbling air (purified by passing through concentrated sulphuric acid and Ascarite) through the solution at 60° ± 5° C. for 0·5 hr. Filter, and wash the precipitate with warm water until the volume of the filtrate is 250 ml. Cool the solution and add carbonate-free 0·05 *N* sodium hydroxide until the *p*H is 6·30. Add 40 g. of mannitol and titrate with the 0·05 *N* sodium hydroxide until the *p*H is again 6·30. The amount of alkali used in the titration, corrected as described below, is a measure of the boric oxide. A double precipitation is sometimes necessary to recover co-precipitated boric oxide. Dissolve the precipitate in 10 ml. of 6 *N* hydrochloric acid, repeat the above procedure, and titrate the second filtrate separately.

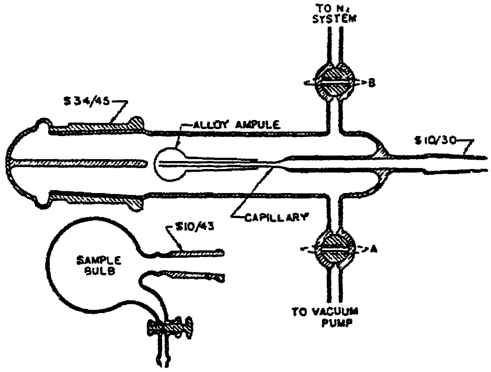
(*B*) *Using an indicator*—Add 20 drops of 0·04 per cent. bromocresol purple solution to the solution of the melt. Add 6 *N* sodium hydroxide until the first distinct colour change (yellow to dirty green) can be seen. This indicates a *p*H of about 5·5. Continue by the above method, adjusting the *p*H to 6·30 with the aid of a phosphate comparison buffer solution.

Titration corrections—In the first adjustment of the *p*H to 6·30, a small amount of boric acid is neutralised and in the second the equivalence point is not quite reached. Under the above conditions, which are selected to give the minimum correction, the amounts of boric acid shown in the table must be added to the amount titrated. These corrections are based on potentiometric titrations of boric acid under the conditions described.

H_3BO_3 found, mg.-equivalents	Correction, mg.-equivalents	H_3BO_3 found, mg.-equivalents	Correction, mg.-equivalents
0·0	0·000	1·2	0·024
0·1	0·005	1·4	0·026
0·2	0·008	1·6	0·029
0·4	0·013	1·8	0·031
0·6	0·016	2·0	0·033
0·8	0·019	2·4	0·036
1·0	0·022	2·8	0·040

Small amounts of carbon dioxide and silica do not interfere with the titration as the *p*H is the same before and after it. Larger amounts make the end-points indeterminate, but are removed if the above method is followed. Aluminium, iron, calcium, barium, magnesium, potassium, lithium, lead, zinc, manganese, antimony, arsenic, titanium, zirconium, sulphur, and fluorine do not interfere. Phosphate causes high results unless the melt is dissolved in 6 *N* nitric acid and the phosphate precipitated by adding a slight excess of silver nitrate at *p*H 5·5. L. A. D.

Routine Analysis of Sodium-potassium Alloys. S. L. Walters and R. R. Miller (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 468–469)—The method is rapid and accurate for alloys containing little or no impurities; its main difficulty lies in obtaining a clean, oxide-free sample of known weight.



Apparatus and procedure—To obtain a weighed, oxide-free sample with the apparatus shown, draw about 30 g. of the alloy from its container into the sample bulb by applying suction at the stop-cock, attach the sample bulb to the sampling device, which contains a thin-glass ampoule of known weight, and is supported to allow rotation; withdraw the air through A and refill through B with re-purified nitrogen. Warm the sample bulb and sampler to a temperature above the melting-point of the alloy, and tilt the apparatus so that, on applying suction at A, a small portion of the alloy is drawn into the ampoule. Re-admit nitrogen to force the excess of alloy from the capillary back to the sample bulb. Return the apparatus to the horizontal position, withdraw the ampoule and seal it in a flame; weigh after cooling. With a glass rod, break the ampoule under neohexane that has been washed first with sulphuric acid and then with alkali before distillation over sodium. Add ethyl alcohol dropwise to decompose the sample, the vessel being submerged in a water-bath to minimise over-heating. When decomposition is complete, transfer the liquid to a 250-ml. Erlenmeyer flask, and titrate with N hydrochloric acid to the phenolphthalein end-point. Then evaporate off the hexane and continue the titration to the methyl orange end-point. By this means the danger of over-titrating is minimised, and the time of titration accordingly reduced.

Calculation—If the potassium content of a W-g. sample of the alloy is X g., and the titre to methyl orange is $M.E.$ mg.-equivalents, then

$$M.E./1000 = X/39{\cdot}1 \quad + \quad (W-X)/23,$$

whence $$X = 2{\cdot}43W - 0{\cdot}0559M.E.$$

The method gives an accuracy of 0·1 per cent. of potassium, and less than an hour is required for a titration. Errors are incurred by the use of impure hexane, by the presence of an oxide film on the sample, and by loss of sample during the transference.

Solid alloys—The freezing point may be conveniently used for identifying the composition of alloys containing more than 92 per cent. of potassium. Using a magnesia cooling-bath and plotting freezing point against the alloy composition as determined by the above volumetric method, a straight line, represented by the equation $Y = 0{\cdot}259t + 83{\cdot}5$, where Y is the percentage by weight of potassium, and t is the Centigrade temperature, was obtained in the region 92 to 100 per cent. of potassium. The freezing point of potassium of purity greater than 99·0 per cent. has been determined as 63·7° C. M. E. D.

Determination of Rates of De-oxidation of Iron Oxide Materials in Reducing Gases. E. P. Barrett and C. E. Wood (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 285–286)—The rate of reduction of iron oxide is of importance in work such as a study of the relation between the porosity of an iron ore, its relative reducibility, and the size to which it should be crushed for efficient use in a blast furnace (T. L. Joseph, *A.I.M.M.E., Tech. Publ.*, 688, 1936). It is usual to determine the rate by heating a sample in a tube furnace in a stream of hydrogen, absorbing the water produced, and weighing it at regular intervals (T. L. Joseph, E. P. Barrett, and C. E. Wood, *Blast Furnace Steel Plant*, 1933, **21**, 147–150, 207–210, 260–263, 321–323, and 336). The new method is claimed to have several advantages. The sample is heated in a current of hydrogen or other reducing gas while it is suspended from a balance, so that the loss in weight may be determined at any time during the process.

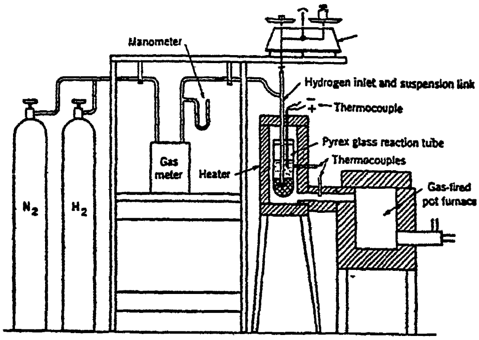


Fig. 1.

A hydrogen inlet tube of heat-resisting alloy, with a distributor on its lower end, extends to the bottom of the sample tube, which resembles a test-tube, 1·7″ × 12″, of Pyrex, or heat-resisting alloy.

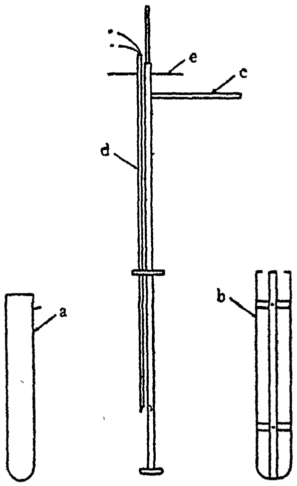


Fig. 2. Parts of Loss-in-Weight Apparatus.

a. Reaction tube
b. Stainless Steel Basket
c. Hydrogen inlet and connection to balance
d. Thermocouple
e. Heat deflector

A 2″-layer of −10 to +14 mesh mullite grog in the bottom of the tube serves to heat and further to distribute the hydrogen. The sample tube is supported by a stainless steel basket fastened to the inlet tube. The assembly is suspended from one side of a balance by a rod and is enclosed by a furnace. A water-cooled plate is fitted between the furnace and the balance. Gas is fed to the inlet tube through a gas meter and a manometer is also fitted.

Preparation of the sample—Sieve through a 0·525″ mesh, crush oversize material to pass the sieve, and add it to the undersize product. Wet-screen on 20-mesh, dry the oversize at 105° C. and separate into four sizes:—0·525″ to +3 mesh; −3 to +6 mesh; −6 to +10 mesh; and −10 to + 20mesh. Make a 227-g. (0·5 pound) composite sample of the four portions.

Procedure—Suspend the charge in the furnace and pass nitrogen through the inlet tube. When the desired temperature is reached stop the nitrogen flow and pass in hydrogen, *e.g.*, at a rate of 0·25 cu.ft. per min. Record the loss in weight at 5-min. intervals until constant weight is obtained. The loss of weight during the passage of nitrogen represents the loss on ignition at the temperature of the test. Determine the iron and manganese contents of a duplicate composite sample to check the available oxygen. L. A. D.

Colorimetric Determination of Antimony. A. I. Kokorin (*Zavod. Lab.*, 1946, **12**, 64–68)—Tervalent antimony in dilute sulphuric acid gives with phosphomolybdic acid a yellow-green colour that turns blue (*cf.* Feigl and Neuber, *Z. anal. Chem.*, 1923, **62**, 376) when sufficient sulphuric acid is added to decompose the excess of reagent. Application of the reaction to the determination of small amounts of antimony in, *e.g.*, copper and tin, is now described.

With 0·15 mg. of antimony and 2 ml. of a 5 per cent. aqueous solution of phosphomolybdic acid in a final volume of 30 ml., which includes 8 ml. of 7 *N* sulphuric acid used for destroying the excess of reagent, the acidity during the 10- to 15-minute reduction (over boiling water) must not exceed 0·5 *N*. An antimony-free solution becomes colourless when the final acidity exceeds 1·5 *N*, but this acidity must not exceed 2 *N* in actual determinations or the colour intensity of the blue reduction product will be lowered. Excess of phosphomolybdic acid has no effect. With an acidity of 0·4 *N* during the reduction, the colour intensity of the final solution attains its full value when the time of heating on the water-bath has been not less than 10 min. Quantities of stannic tin up to twice the amount of antimony present, and of cupric copper up to 1 mg. in 50 ml., have no effect. Iron in any form interferes. Bismuth up to 50 mg. in 50 ml. has no effect. Large quantities of bismuth tend to counteract the adverse effect of higher acidities on the reduction, which may then be carried out in *N* acid without loss of colour intensity in the final solution.

Procedure—Prepare standard solutions for use with a photo-colorimeter as follows:—Dissolve 0·1 g. of A.R. antimony metal in 50 ml. of concentrated sulphuric acid by heating, pour into 500 ml. of water in a 1-litre graduated flask, add 50 ml. of sulphuric acid, cool, and make up to the mark. By means of a micro-burette, run various volumes (0·5 ml. and greater) into conical flasks. To each add sufficient 7 *N* sulphuric acid or alkali to make the acidity 0·4 *N* on dilution with water to 30 ml. After dilution, add 2 to 3 ml. of a saturated aqueous solution of sulphur dioxide, boil to remove sulphur dioxide, dilute again to 30 ml., add 2 ml. of freshly prepared, 5 per cent. phosphomolybdic acid solution, and heat for 10 min. over boiling water. Cool to room temperature, add 8 ml. of 7 *N* sulphuric acid, shake periodically for 5 min., transfer to a 50-ml. measuring flask, and make up to volume with water. Measure the colour intensity by means of a compensation photo-colorimeter, yellow filters being used. In solutions so obtained, 0·05 to 0·5 mg. of antimony in 50 ml. can be accurately determined.

To determine antimony in tin and copper, take 0·1 to 1 g. of tin or 2·5 g. of copper and deposit the antimony on a copper foil spiral (*cf.* Clarke, ANALYST, 1928, **53**, 373), place the washed foil in a small beaker, add 25 ml. of 0·5 *N* sodium hydroxide and 15 drops of perhydrol, and boil for 5 min. Transfer the solution to a conical flask, rinse the foil and the beaker two or three times with the minimum amount of water, add 1·5 ml. of concentrated sulphuric acid, and 3 ml. of saturated sulphur dioxide solution, and evaporate until fumes just appear. Add 15 ml. of water, 14 ml. of 2 *N* sodium hydroxide, then 2 ml. of phosphomolybdic acid, and proceed as above. Results are accurate to about 1 part in 10. G. S. S.

Amperometric Titration of Cyanide with Silver Nitrate, using the Rotating Platinum Electrode. H. A. Laitinen, W. P. Jennings, and T. D. Parks (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 574–575)—With a rotating platinum electrode, the end-point of the amperometric titration of cyanide by silver nitrate can be obtained with an accuracy of 0·1 to 0·2 per cent. for cyanide concentrations as low as 0·002 *N*, which is comparable in accuracy with the visual (Dénigès) end-point. The visual end-point becomes indistinct at 2×10^{-4} *N* cyanide, and fails at higher dilutions, whereas the amperometric end-point is still observed when 4×10^{-6} *N* cyanide is titrated with 5×10^{-4} *N* silver nitrate.

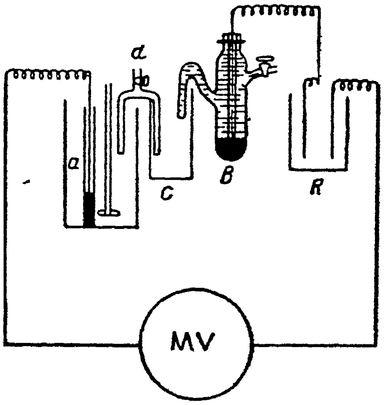
The normality of the silver nitrate solution should be, in general, approximately five times that of the cyanide solution, so as to avoid the necessity of correcting for the dilution effect, but should be not less than 5×10^{-4} *N*. Between titrations, silver must be removed from the platinum electrode either by anodic polarisation or by means of nitric acid. If nitric acid is used, the electrode must stand in aqueous ammonia solution for a few minutes and be rinsed with water before use.

Titration results are accurate to 0·5 per cent. in the presence of a fifty-fold excess of chloride or bromide, or in 1 *N* salt solutions. High alkali concentrations tend to give high results. Compounds that form very insoluble silver salts, *e.g.*, iodides or sulphides, or sulphydryl compounds, or materials that form, with silver, complexes more stable than that formed by cyanide will, in general, interfere with the method. J. G. W.

Potentiometric Methods of Determining Aluminium in Magnesium Alloys of Elektron Type. L. J. Poljak (*Zavod. Lab.*, 1946, **12**, 268–275)—Potentiometric methods of titrating aluminium with (*a*) sodium fluoride, using a platinum electrode in a ferrous - ferric solution, and (*b*) sodium hydroxide, using an antimony electrode, are studied.

With (*a*), aluminium forms a complex $[AlF_6]'''$ that is much more stable than the corresponding iron complex, so that as long as aluminium ions are present the sodium fluoride added should go to form Na_3AlF_6 and the oxidation-reduction potential determined by the ratio $Fe^{\cdot\cdot\cdot}$: $Fe^{\cdot\cdot}$ should scarcely change until all the aluminium ions are removed and the iron complex starts to form. At this point, the potential should change suddenly. The method, as described by Treadwell and Bernasconi (*Helv. Chim. Acta*, 1925, **13**, 500) is, however, unsatisfactory, since the potential change at the end-point is poorly defined and the electrode process is so slow that the titration takes 40 to 50 min. A modification (the addition of a larger amount of ferric salt) due to Tarajan (*Ibid.*, 1939, 8, 3) gives a sharper change and reduces the titration time to 10 to 15 min. The method has now been further improved and applied satisfactorily to the analysis of magnesium alloys containing 2 to 10 per cent. of aluminium. The addition of dilute acid at the stage in the titration where the potential begins to fail, *i.e.*, when about 1/3 to 1/4 of the aluminium has reacted, causes the potential of the indicator electrode to return to its earlier value and to remain constant until the equivalence point is reached, as shown by a sharp fall. Aluminium may be determined thus in presence of iron, titanium, silica, zinc, manganese, small quantities of nickel and cadmium, and a ten-fold excess of magnesium. Copper interferes, but it may be separated from aluminium by precipitation of the latter with aqueous ammonia, which serves also to remove the bulk of magnesium.

Procedure for (*a*)—Dissolve 1 g. of the alloy in 30 ml. of diluted hydrochloric acid (1 + 1), precipitate aluminium hydroxide with aqueous ammonia using methyl red as indicator, filter but do not wash, transfer the precipitate to the original beaker, wash the filter with 30 to 40 ml. of hot, diluted hydrochloric acid (1+1), and heat to dissolve the hydroxides. Cool, make up to 100 ml. in a measuring flask, and pipette 25 ml. into the titration beaker, *a*. This is fitted with an ebonite cover through which pass the indicator electrode (*cf.* Treadwell, *loc. cit.*), the burette jet, a glass stirrer, a glass tube for introducing carbon dioxide from a cylinder, and one arm of an inverted U-tube containing saturated potassium chloride solution, which serves to connect the solution in the beaker with a saturated calomel electrode. A millivoltmeter, MV (reading 18 millivolts, internal resistance 840 cm.) is connected on the one side to the indicator electrode, and on the other to the calomel electrode, *B*, through a variable resistance, *R* (0·05 *N* copper sulphate in a beaker containing one fixed copper wire and one movable copper wire in a glass sheath, arranged so that the resistance may be regulated before the test by moving the latter in or out of the solution). Add to the solution under test 0·5 ml. of 0·05 *N* ferric chloride or sulphate, neutralise to tropaeolin OO with aqueous ammonia (the optimum *p*H for the reaction is 2 to 2·5), saturate the solution with sodium chloride, and add an equal volume of ethyl alcohol. Then pass carbon dioxide gas for 2 to 3 min., introduce 2 to 3 drops of cold, freshly prepared, saturated ferrous sulphate solution, and titrate with *N* sodium fluoride, standardised potentiometrically on aluminium chloride, without stopping the current of carbon dioxide during the whole of the titration. When the potential starts to fall, add a few drops of very dilute acid and continue the titration to the sharp end-point. To standardise the fluoride solution, take 10 ml. of aluminium chloride solution containing 3 g. of aluminium per litre, add 0·5 ml. of 0·05 *N* ferric sulphate, dilute with water to 25 to 30 ml., neutralise, and titrate as described above. Results are satisfactory, *e.g.*, with Elektron type alloys (chemical results in brackets), the values 7·47, 7·44, 7·53 (7·43) and 3·46, 3·46, 3·55 (3·50), were obtained.



With (*b*), in spite of adverse criticisms by previous workers (Treadwell *et al.*, *loc. cit.*; Stefanovski, *J. Applied Chem. Russ.*, 1932, **5**, 92, etc.), it is shown that the method may be made suitable for routine analysis of magnesium alloys. Experimental errors do not exceed 3 per cent., the method is economical in materials, and it takes only 20 min. in all.

Procedure for (*b*)—Place a suitable weight of the sample (3 g. for 2 per cent., 1·5 g. for 4 per cent., 1 g. for 6 to 8 per cent., or 0·5 g. for 10 to 12 per cent. of aluminium) in a 250-ml. conical flask, cover with 25 ml. of water, and add in small portions, avoiding unnecessary excess, 8 to 10 ml. of concentrated hydrochloric acid. After dissolution of the alloy, add 3·5 g. of ammonium chloride per g. of alloy taken, dilute somewhat with water, and neutralise to bromophenol blue (2 to 3 drops of 0·2 per cent. aqueous solution). (Potentiometric titration of the acid is not satisfactory since the potential change at neutrality is indefinite). Transfer the solution to the titration beaker (the apparatus set-up is the same as for the fluoride titration, except that an antimony electrode, in the form of a cast rod, polished with fine emery paper and then with filter paper, the polishing being repeated after every 3 to 4 tests, takes the place of the platinum), and titrate with 0·5 to 0·7 *N* sodium hydroxide, carbonate-free, that has been standardised potentiometrically against oxalic acid or aluminium chloride, adding the alkali in 0·2-ml. portions close to the equivalence point, which is indicated by a sudden potential rise. Copper interferes, but in Elektron type alloys it can normally be ignored. If an insoluble residue appears after the acid attack on the alloy, filter before adding ammonium chloride to avoid dissolving more than traces of copper. G. S. S.

Agricultural

Chromatographic Determination of Carotene J. B. Wilkes (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 702–707)—The chromatographic technique has been extensively used for the quantitative separation of carotene from other plant pigments; powdered sugar, soda-ash, and mixtures of magnesium oxide with soda ash or kieselguhr have been proposed as adsorbents for this purpose. Heat-treated kieselguhr, especially Johns–Manville Hyflo Super-Cel, has been found to be an excellent adsorbent and its use is now recommended. Any of the well-known methods of extracting plant pigments with hydrocarbon solvents may be used, but polar solvents must be removed before chromatography. The method is suitable for the preparation of carotene from kryptoxanthin, xanthophyll esters, and acid-decomposition products of xanthophyll. It does not enable carotene to be separated from lycopene.

Procedure—Extraction of carotene—Weigh into a 125-ml. conical flask 1 to 2 g. of finely ground (40-mesh) alfalfa meal, and cover with a mixture of 20 ml. of redistilled acetone and 40 ml. of light petroleum (b.p. 30° to 60° C.) Stopper tightly and leave the flasks in the dark for 16 to 70 hours. Decant the liquid into a 300-ml. beaker, rinse out the flask with two 50-ml. portions of light petroleum, and evaporate to 10 to 15 ml. on a water-bath. Add a further 50 ml. of light petroleum, again evaporate to 10 to 15 ml., and add a further 40 ml. of light petroleum. This procedure removes all traces of acetone and the solution is ready for chromatography. For routine control work, a simplified method may be used. Weigh a 1-g. sample of the finely-ground meal into a 125-ml. conical flask. Add 100 ml. of light petroleum (b.p. 30° to 60° C.), stopper tightly, and leave in the dark for at least 16 hours.

Chromatographic Separation of Carotene—Fill a glass column, 3·3 cm. in diameter, to a height of 15 to 25 cm. with Hyflo Super-Cel. The most rapid method is to insert a plug of cotton wool in the bottom of the tube and then force the tube into a container of the adsorbent. When sufficient adsorbent has been introduced, press it firmly down with a plunger. For more exacting separations, add the adsorbent in small quantities at a time and press the material firmly with a plunger after each addition. Apply suction to the column and pour the extract, without filtering it, on to the top of the column. Wash the carotene through the column with light petroleum until the filtrate is colourless. Make up the solution to 200 ml. and estimate the carotene colorimetrically. When the material contains appreciable quantities of fat, a longer column is required.

Estimation of vitamin A—Vitamin A ester, like carotene, is only feebly adsorbed on Hyflo Super-Cel whereas free vitamin A is more strongly adsorbed. The two forms of vitamin A give separate bands that fluoresce when exposed to ultra-violet light. The method appears to have a possible application in the estimation of vitamin A.

Extraction with a mixture of light petroleum and purified acetone gave higher results than extraction with light petroleum alone, but with impure acetone low results were obtained, owing to loss by oxidation; it is suggested that addition of dimethylaniline or hydroquinone would prevent this. If a trace of acetone is left in the petroleum extract, a grey band, having an orange-red fluorescence in ultra-violet light, will pass into the carotene band; this grey band is normally retained at the top of the column. A 4 per cent. solution of acetone in light petroleum may be used to separate the non-carotenoid pigments from the chromatogram, if desired. W. S. W.

Gas Analysis

Determination of Oxides of Sulphur in Flue Gas. E. W. F. Gillham (*J. Soc. Chem. Ind.*, 1946, **65**, 370–372)—Existing methods for the determination of sulphur dioxide and sulphur trioxide are discussed. A common disadvantage is the use of alkaline absorbents in which sufficient oxidation of sulphite occurs to cause large errors in the trioxide result, even when inhibitors are used. The method described uses distilled water as absorbent and gives more accurate results.

Apparatus—This consists of two bubblers connected in series, a gas volume meter, and any suitable pump. The first bubbler is equipped with a sintered-glass dispersion disc of No. 1 porosity and contains 50 ml. of distilled water. This absorbs all the sulphur trioxide up to about 0·2 grain per cu.ft., some dioxide, and other acid constituents such as oxides of nitrogen. The second bubbler is a plain one and is charged with 10 ml. of neutral, sulphate-free, 20-volume hydrogen peroxide, and 40 ml. of water. The water-sealed gas meter is placed after the absorption train. Flue gas is drawn through the apparatus at a rate of 0·5 to 1 cu.ft. per hour (15 to 30 litres per hour), preferably through a sampling tube of not less than 5 mm. bore to avoid carrying grit into the absorbers.

Reagents—*Screened methyl red indicator*—Dissolve 0·4 g. of methyl red in 15·2 ml. of 0·1 N sodium hydroxide: dissolve 0·2 g. of methylene blue in water. Mix and dilute to 1 litre. *Phenolphthalein - thymol blue indicator*—Dissolve 0·2 g. of thymol blue in 250 ml. of water and 250 ml. of alcohol: dissolve 1·25 g. of phenolphthalein in 250 ml. of alcohol. Mix and dilute to 1 litre with water. *Standard potassium palmitate solution*—Dissolve 25·63 g. of palmitic acid by warming with 250 g. of glycerol and 400 ml. of alcohol. Add 5 ml. of phenolphthalein solution, and alcoholic potassium hydroxide solution (34 g. per litre) until the solution is just coloured pink. Cool, dilute to 1 litre with 90 per cent. alcohol, and standardise against 0·1 N barium chloride.

Procedure—Rinse the contents of the bubblers into flasks. Titrate the solution from the first bubbler with 0·1 N sodium hydroxide (a ml.), using screened methyl red indicator. Add a few drops of hydrogen peroxide and titrate again (b ml., representing the sulphur dioxide in the first bubbler). Titrate the contents of the second bubbler (c ml.). Add a few drops of nitric acid to each solution, boil, and add a measured volume of 0·1 N barium chloride sufficient to precipitate all the sulphate and leave an excess of about 10 ml. Boil for 10 min., cool rapidly, and determine the excess of barium chloride by titrating with the palmitate solution as follows. Add 1 ml. of phenolphthalein - thymol blue indicator and then add N sodium hydroxide until the colour changes to green. Add 0·1 N sodium hydroxide until the colour just becomes violet and discharge this colour by adding a drop of 0·1 N nitric acid. Titrate with the palmitate reagent until the violet colour again appears. If the palmitate equivalent of the sulphate in the first bubbler is d ml. of 0·1 N solution and of the second bubbler e ml. then:

Sulphur trioxide absorbed $\equiv d-2b$ ml. of 0·1 N solution
Sulphur dioxide „ $\equiv e+2b$ „ „
Other acids „ $\equiv a+b-d+c-e$ „

L. A. D.

Automatic Determination of Sulphur Trioxide in Flue Gas. E. W. F. Gillham (*J. Soc. Chem. Ind.*, 1946, **65**, 405–409)—The flue gas is scrubbed by being passed through a wetted sintered-glass disc. All the sulphur trioxide and some sulphur dioxide are absorbed. The solution of sulphur oxides is then scrubbed with flue gas that has been freed from acid gases and the sulphur dioxide is thereby removed. The electrical conductivity of the solution is then determined and used as a measure of the sulphur trioxide content.

Apparatus is described in which these operations are carried out continuously so that a record of sulphur trioxide concentration is obtained. Devices are incorporated to ensure constant conditions in the apparatus under all conditions of plant load and flue-gas pressure. The original paper should be consulted for full details and diagrams.

L. A. D.

Water Analysis

New Simple Titration Methods for Determination of Hardness of Water. G. Schwarzenbach, W. Biedermann, and F. Bangerter (*Helv. Chim. Acta*, 1946, **29**, 811–818)—In earlier communications (*Idem.*, 1945, **28**, 181, 377, 828, 1133; 1946, **29**, 364), it was stated that certain derivatives of imino-diacetic acid are suitable for determinations of hardness of water. Four methods for the application of these derivatives are now described.

Method A—The derivative used in this method is disodium ethylenediamine tetra-acetate, and the standard solution (0·1 M) is made by dissolving 37·21 g. of the salt ($Na_2C_{10}H_{14}O_8N_2.2H_2O$) in a litre of water. Each millilitre of this solution is equivalent to 1 mg.-ion of calcium. A saturated solution of murexide (ammonium purpurate) serves as an excellent indicator for the calcium ion in alkaline solution. Treat 200 ml. of the water with 10 ml. of N sodium hydroxide and with 0·3 ml. of the indicator and titrate immediately with the standard disodium ethylenediamine tetra-acetate solution. It is inadvisable to allow the alkaline solution to stand long before titration since calcium carbonate may then be precipitated. The end-point occurs within three drops and the best results are obtained by taking the mid-point of the colour change. Murexide, although affected by zinc, cadmium, mercuric, and cupric ions, as well as by the calcium ion, is not affected by the magnesium ion, and calcium can therefore be determined in presence of magnesium.

Method B—In this method the same standard solution of the complex salt as in Method A is used. The indicator is a mixture of 2 parts of methyl red and 3 parts of bromocresol green dissolved to a concentration of 0·2 per cent. in alcohol. To 200 ml. of the water add 15 drops of the indicator and with very dilute hydrochloric acid adjust the colour to the turning point of the indicator, which is at *p*H 5. As a comparison solution use 190 ml. of distilled water with 10 ml. of the standard complex salt solution and 15 drops of indicator. To the sample solution now add 10 ml. of the standard complex salt solution and titrate the mixture, now become acid to the indicator, back to *p*H 5 with 0·1 *N* sodium hydroxide which has been standardised against the derivative, each g.-molecule of the complex salt being equivalent to 2 g.-molecules of sodium hydroxide. This method has the advantage that the temporary hardness and permanent hardness may be determined successively. The temporary hardness is found by titrating the water sample to *p*H 5. The carbon dioxide is then expelled, the standard complex salt solution is added, and the sample is titrated back to *p*H 5 to obtain the remaining hardness. The method is subject, however, to a slight error due partly to the somewhat indistinct end-point and partly to the stoicheiometric end-point's being at *p*H 5·3 and not at *p*H 5. With practice, however, good results can be obtained. The method cannot be used in presence of magnesium.

Method C—In this method the trisodium salt of ethylenediamine tetra-acetic acid is used. To prepare the standard solution dissolve 37·21 g. of disodium ethylenediamine tetra-acetate in water, add 0·1 molecular equivalent of sodium hydroxide and dilute to 1 litre. The end-point is at *p*H 8·5 and the indicator used is 0·1 per cent. alcoholic phenolphthalein. To prepare the comparison solution add 10 ml. of the standard complex salt solution to 200 ml. of boiled and cooled distilled water, add 10 drops of indicator, and titrate to a faint permanent pink colour with 0·1 *N* sodium hydroxide. Record the number of millilitres required. Slightly acidify 200 ml. of the water to be tested, remove carbon dioxide by boiling, cool, add 10 drops of indicator and titrate to the pink colour of the comparison solution. Now add 10 ml. of the complex salt solution and again titrate to the colour of the comparison solution. From the titre of the sample deduct the titre of the comparison solution. Each g.-equivalent of base corresponds to 1 g.-ion of calcium or magnesium. By this method the sum of the calcium and magnesium contents is titrated; the procedure is thus useful for the determination of total hardness. Removal of carbon dioxide sharpens the end-point, but is not absolutely necessary.

Method D—The complex salt used in this method is the neutral sodium salt of uramil diacetic acid. To prepare the standard solution dissolve 27·71 g. of pure uramil diacetic acid in water, neutralise with sodium hydroxide solution to methyl red indicator, and dilute to 1 litre. To prepare the comparison colour solution at *p*H 5·5 add to 200 ml. of distilled water 14 ml. of *N* sodium acetate solution, 2 ml. of *N* acetic acid and 10 drops of the indicator, which is the methyl red and bromocresol green mixture used in Method B. Treat 200 ml. of the water to be tested with 10 drops of indicator and with hydrochloric acid or sodium hydroxide until the colour is that of the comparison solution. Add 10 ml. of the sodium uramil diacetate solution and titrate with 0·1 *N* sodium hydroxide solution to the comparison colour. When magnesium is present this method gives the sum of the calcium and magnesium contents and is thus useful for the determination of total hardness. It has the advantage over Method C that, since the determination is in an acid medium (*p*H 5·5), carbon dioxide has no effect.

Methods A and C are to be recommended since they give the lime hardness and the total hardness, respectively. Method B is somewhat less accurate and is suitable only when magnesium is absent. Method D gives good results, but uramil diacetic acid is not available commercially. The complex substances mentioned are obtainable from the firm of Siegfried in Zofingen.

A. O. J.

Physical Methods, Apparatus, etc.

Most Economical Sampling for Chemical Analysis. C. W. Churchman (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 267–268)—The method of sequential analysis developed by A. Wald (*Ann. Math. Stat.* XVI, 1945, 118, and *J. Amer. Stat. Assoc.*, 1945, **40**, 277) is applied to the problem of discriminating with as few analyses as possible between alternative formulae for organic compounds. In the simplest case only one element, α, is being estimated and, on the hypotheses that the compound has the formula 1 or 2, the percentage of this element will be α_1 or α_2. It is assumed that σ_α^2, the error variance of a single determination, is already known. $\overset{n}{\Sigma}\alpha$ denotes the sum of the first n determinations of α. Calculate $h = \frac{4{\cdot}595}{\alpha_2 - \alpha_1} \sigma_\alpha^2$ and $s = (\alpha_1 + \alpha_2)/2$, where $\alpha_2 > \alpha_1$. The hypothesis that the true percentage α is α_1 can be accepted as soon as $\overset{n}{\Sigma}\alpha \leq sn - h$, and the alternative hypothesis that the true percentage is α_2 can be accepted as soon as $\overset{n}{\Sigma}\alpha \geq sn + h$. For $\overset{n}{\Sigma}\alpha$ lying between $sn - h$ and $sn + h$, it is necessary to take further observations. The constant 4·595 used above refers to the 0·01 probability; this implies that the probability of accepting possibility X when Y is actually true is 0·01, *i.e.*, we go wrong in this way one time in 100. Similarly, the chance of accepting possibility Y when possibility X is actually true is 0·01: for the 0·001 and 0·05 levels of probability, the corresponding constants are 6·907 and 2·944.

The more complicated case of two elements α and β being determined is also dealt with. If n and m are the numbers of determinations of α and β, respectively, the critical function is

$$\left[\frac{\alpha_2-\alpha_1}{\sigma_\alpha^2}\right]\overset{n}{\Sigma}\alpha + \left[\frac{\beta_2-\beta_1}{\sigma_\beta^2}\right]\overset{m}{\Sigma}\beta - n\left[\frac{\alpha_2^2-\alpha_1^2}{2\sigma_\alpha^2}\right] - m\left[\frac{\beta_2^2-\beta_1^2}{2\sigma_\beta^2}\right]$$

and should this be less than or equal to $-4{\cdot}595$ we accept the hypothesis that $\alpha = \alpha_1$, and $\beta = \beta_1$; should it be greater than $+4{\cdot}595$ we accept $\alpha = \alpha_2$, and $\beta = \beta_2$; for intermediate values more observations are necessary. The procedure depends on our previous knowledge of the error variances, and is then the most efficient available.

An example of the application of this method in deciding between the two formulae $C_{19}H_{12}O_6Br_4S$ and $C_{19}H_{10}O_5Br_4S$ from experimental determinations of the sulphur and bromine contents of a compound is given. K. A. B.

Improvement of Precision by Repeated Measurements. Application to Analytical Control Methods. J. Mandel (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 280–284)—The practice of duplicating analyses is well established among analysts, but it is well known that frequently the apparent closeness of the duplicate gives a grossly misleading idea of the accuracy of the analysis. Moran (*Ibid.*, 1943, **15**, 361–364) ascribes this to the fact that duplicate analyses made at the same time are not truly random, but are rather influenced by slight variation in technique, or surrounding conditions.

The methods of the analysis of variance are applied to estimating a quantity "the coefficient of improvement by N-fold replication" (*i.e.*, by carrying out the analysis N times on a particular occasion) defined by $(CI)_N$ = (variance of a single determination)/(variance of the average of N parallel determinations).

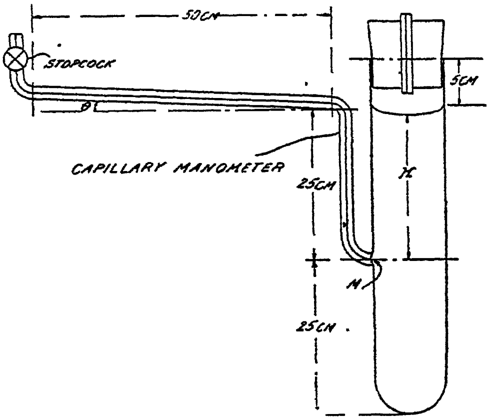
The larger the variability between series, the lower will be $(CI)_N$: when there is no improvement by replication on a given occasion, it will have the limiting value 1; and when there is complete independence of the replication on a given occasion, it will be equal to N. It is shown how to lay down confidence limits for the estimated value for $(CI)_N$.

These methods are applied to discussing the effect on the over-all error of varying M and N where we have M series (at different times) of N analyses (at the same time). The case is also discussed where a blank determination on a standard is done at the same time.

Data obtained in routine determinations over a period of one year for the specific gravity of carbon tetrachloride, and the data obtained by Williams and Haines (*Ibid.*, 1944, **16**, 157) in their new method of determining sodium in potassium hydroxide are used to illustrate the application of the methods indicated above. K. A. B.

Measuring the Distribution of Particle Size in Dispersed Systems. W. M. Dotts (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 326–328)—The method is based on the micro-manometric procedure, whereby the change in concentration of the dispersed internal phase during sedimentation is traced by the change in pressure at a fixed point below the surface of the suspension. The simplified procedure described is suitable for all systems from concentrated emulsions to dilute suspensions. Systems of mixed pigments where photographic or colorimetric methods give dubious results are readily tested by this method, which is also suggested for measurements upon emulsions of all concentrations at various ages to determine the effects of ageing upon the distribution of globule size. In order to avoid the effects of hindered settling, concentrated suspensions or emulsions should be diluted before measurement to a concentration of about 5 per cent. by volume of internal phase. According to the author, most emulsions having a concentration of internal phase not greater than the close-packing ratio for equal spheres (74·048 per cent.) do not break on dilution with excess of the pure external phase, and there is little evidence that dilution affects the size of the dispersed globules. The testing of a series of similar emulsions under approximately the same conditions will always tend to increase the significance of the comparative results.

Constructional details—The apparatus is shown in the figure. The capillary should have a uniform inside diameter of about 0·75 mm. and an outside diameter of 8 mm. for rigidity. Tubing of smaller bore should not be used owing to the effects of capillarity. The length of the manometer gauge should be 50 cm., and sin θ, where θ is the angle with the horizontal, should lie between 0·01 and 0·05 for most emulsions, while a value of about 0·1 is suitable for most dispersions. If any difficulty arises in obtaining the desired value of sin θ, a trial run can be made during which the clamps may be adjusted to bring the manometer as close as possible to the horizontal as necessary to obtain the desired accuracy. The position of the meniscus may conveniently be read by means of a millimetre scale fixed to the manometer tube. Since this position may be measured to the nearest 0·1 mm. with simple equipment, a precision of 1 per cent. is obtained on all but 2·5 per cent. or less of the dispersed phase. The diameter of the sedimentation vessel should not exceed 16 mm. and it should be long enough to contain 50 ml. below and above *M*, the point of junction of the manometer. The height *H* of the liquid surface above the point *M* should also be known to the nearest millimetre.



Procedure—Set up the apparatus in a room or bath where a fixed temperature can be maintained to ± 0·5° C., and fill the manometer tube with dispersion medium, leaving the main tube empty. Pour the suspension under test, suitably diluted, into the main tube to a pre-determined mark such that the meniscus in the manometer tube is supported near the beginning of the scale close to the stop-cock. Start the timer immediately, and simultaneously open the stop-cock. Take the first few readings at close intervals so that the position of the meniscus at zero time may be obtained by a short extrapolation. The rate of recession of the meniscus due to evaporation *via* the stop-cock and the main tube should be determined for each liquid used. Volatile liquids will give a recession of only a fraction of a millimetre per hour. Continue to read the position of the meniscus at suitable intervals until the rate of recession approaches this value.

Computation of results—If Y represents the total recession of the meniscus in the manometer during a run, y the recession after time t, and P the percentage of the total mass of internal phase settling or rising past the point M during the same time, it can be shown that $P = 100y/Y$. The actual material represented by any value of P is a heterogeneous system consisting of (*a*) particles in size groups the whole of which have passed M during the time t, and (*b*) smaller particles belonging to all the other size groups present. The rate of sedimentation (up or down) of all these groups is dP/dt, and $t.dP/dt$ gives the percentage of dispersed phase in these latter size ranges. Therefore, if S represents the percentage of internal phase in class (*a*) above,

$$P = S + t.dP/dt.$$

If P is plotted against t, the ordinate intercept on the P axis of any tangent to the resulting curve will be a value of S (Fischer and Odén, *Proc. Roy. Soc. Edinburgh*, 1924, **44**, 98), and the difference between the ordinate intercepts of any two tangents to this P, t curve gives the percentage ΔS of the total dispersed phase corresponding to the size range $r \ldots\ldots r_n$ calculated from Stokes's Law for the times in question. The distribution of particle size can now be tabulated for all the material in the dispersed phase.

The following form of Stokes's law should be used for this calculation:

$$r^2 = \frac{9\eta H}{2(d_i - d_x)gt}$$

where r = radius of particles moving with constant velocity; η = viscosity of dispersion medium; H = sedimentation depth; d_i = density of internal phase; d_x = density of external phase; g = acceleration due to gravity; t = time of sedimentation.

Experimental curves are given for three runs on identical suspensions of 1·80 per cent. of Fe_3O_4 in diethylene dioxide. The ordinate deviations of the points from the curve are 1 per cent. or less in all but the initial values. This appears to be the limiting accuracy of the method owing to the influence of temperature variations, inertia, evaporation, and contamination of the walls of the manometer.

B. A. S.

Reviews

INDEX TO THE LITERATURE ON SPECTROCHEMICAL ANALYSIS, PART II, 1940 TO 1945. By BOURDON F. SCRIBNER and WILLIAM F. MEGGERS. Pp. 180. Philadelphia: American Society for Testing Materials. 1947. Price $3.00.

In 1940 the American Society for Testing Materials issued an Index to the Literature on Spectrochemical Analysis covering the years 1920 to 1939, in which references were given to a wide range of subjects such as light sources, photographic materials, progress in quantitative analysis and so forth.

The present volume, Part II, is a continuation of this work and in one important respect is of considerably more value in that each reference is now followed by a useful abstract of the original paper. This expansion is particularly welcome since many of the journals in which the originals appeared are difficult of access owing to the War.

A detailed subject index is included and although no author index is provided, this omission is to some extent off-set by the arrangement of the material in alphabetical order of the authors, for each year under review. The compilers and their staff at the National Bureau of Standards are to be congratulated on the production of this useful work of reference.

B.S. Cooper

SUPPLIES OF LABORATORY CHEMICALS

Letters received from members of the Society setting out difficulties they have encountered in obtaining supplies of laboratory chemicals have been forwarded to the Board of Trade.

A reply has been received to the effect that the information supplied has proved of great assistance. It has enabled the Board to take the matter up with chemical suppliers with a view to ameliorating the position. It is understood that in the first place the Board will obtain a fairly detailed statement of the suppliers' difficulties and that subsequent action will depend on what the Board thus learns.

K. A. Williams, *Hon. Secretary.*

REPRINTS OF PROFESSOR HEYROVKÝ'S LECTURE

Reprints of Professor Heyrovský's Lecture on The Fundamental Laws of Polarography, from the June (1947) Analyst, will shortly be obtainable. Orders should be sent, with remittances, to the Editor, The Analyst, 7–8 Idol Lane, London, E.C.3. Price, to members of the Society 1s. 6d.; to non-members 2s.

MICROCHEMISTRY AND PHYSICAL METHODS GROUPS

A Joint Meeting of these two Groups will be held in the University Chemical Laboratories at Cambridge, on Friday, September 26th, 1947. After visits to the Colloid Science Department and Radiochemistry Laboratory a meeting will be held at 4 p.m. in The Chemistry Lecture Theatre, Pembroke Street, when the following short papers on Micro-physical Methods of Analysis will be read and discussed: "Micro-methods for Molecular Weight Determination," by Cecil L. Wilson, M.Sc., Ph.D., F.R.I.C.; "Turbidimetric Methods used in Agricultural Analysis," by J. Tinsley, B.Sc., Ph.D., F.R.I.C.; "Microchemical Applications of Potentiometric Methods," by J. T. Stock, M.Sc., F.R.I.C.; "Micro-analysis, using X-ray Diffraction Technique," by H. P. Rooksby, B.Sc., F.Inst.P.

Further particulars may be obtained from Dr. J. E. Page, Glaxo Laboratories, Greenford, Middlesex.

PHYSICAL METHODS GROUP

The Third Annual General Meeting of the Physical Methods Group will be held in the rooms of the Chemical Society, Burlington House, London, W.1, at 6 p.m., on Tuesday, November 25th, 1947, and will be followed by a lecture on "Electron Microscopy," by Mr. B. S. Cooper.

AUGUST, 1947 Vol. 72, No. 857

THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS
AND OTHER ANALYTICAL CHEMISTS

THE CHEMICAL SOCIETY

The following Address was presented to The Chemical Society by the President, Mr. Lewis Eynon, at the opening ceremony of the Centenary Celebrations, at the Central Hall, Westminster, on Tuesday, July the fifteenth:—

FROM
THE SOCIETY OF PUBLIC ANALYSTS
AND
OTHER ANALYTICAL CHEMISTS
TO
THE CHEMICAL SOCIETY

On the occasion of the Celebration, on July 15th, 1947, of the Centenary of the Foundation of The Chemical Society, The President, Officers, Council and Members of the Society of Public Analysts and Other Analytical Chemists send Fraternal Greetings to the President, Officers, Council and Fellows of The Chemical Society.

We are glad to have this opportunity to express our friendship and goodwill to the oldest Chemical Society in the World which has so signally served the science of chemistry and to offer our best wishes for its continued prosperity.

(*Signed*) LEWIS EYNON (*President*).
G. TAYLOR (*Honorary Treasurer*).
K. A. WILLIAMS (*Honorary Secretary*).
Dated this Fifteenth Day of July
Nineteen Hundred and Forty Seven.

Seal of the
Society of Public Analysts
and Other Analytical Chemists

The Chemical Estimation of Nicotinic Acid in Cereals and other Foods

By EIRENE M. JAMES, F. W. NORRIS, and FRANK WOKES

Chemical methods of estimating nicotinic acid in foods are generally based on the cyanogen bromide reaction, but vary considerably in the procedure adopted to extract the vitamin and to obviate difficulties caused by interfering substances. The rate of development and the stability of the characteristic yellow colour have been found to be affected by the particular aromatic amine employed as well as by certain other factors. In clinical tests British workers[1,2] have tried to eliminate interfering colours by treatment with permanganate, but did not apply this treatment to cereal samples, with certain of which the chemical results were considerably higher than would be expected from biological assays. American workers[3,4,5,6,7] have used adsorption techniques to separate the nicotinic acid from interfering substances and have used different aromatic amines. Their results on wheat and other cereals have been in good agreement with the results of microbiological assays. But difficulty has arisen with a nicotinic acid "precursor" which occurs in different cereals and can be converted into microbiologically active nicotinic acid by acid or alkaline hydrolysis or even by incubation of the aqueous suspension at 37° C. at *p*H 1 or *p*H 8.[8,9,10] Various workers have shown the presence, in different cereals, of a precursor, which is converted into microbiologically active nicotinic acid when the cereals are germinated.[11,12,13]

The object of the present investigation was to study the effects of the various procedures used by different workers and devise a method which when applied to cereals would give satisfactory reproducibility and agreement with microbiological results.

In the last three years, working on a variety of unmalted and malted cereals and cereal products, we have adapted for use with the Spekker photo-electric absorptiometer a modified chemical method, based on the above British and American techniques, which has been submitted to the Chemical Panel of the Society's Vitamin Sub-Committee for trial by the members. The time seems opportune to publish details of this method as at present used, without of course attempting to anticipate the final decision of the Panel. A summary of some of the results was given in a communication read at a meeting of the Biochemical Society in September, 1945.[14]

CHEMICAL METHOD

REAGENTS—

Cyanogen bromide reagent—Prepare a saturated solution of bromine (AnalaR) in distilled water. Cool it below 10° C. and add, drop by drop, an ice-cold 10 per cent. solution of potassium cyanide (AnalaR) until it is just decolorised. Prepare this solution fresh each day and keep it in a cool dark place.

Stock nicotinic acid solution—Dissolve 100 mg. of nicotinic acid in 50 per cent. alcohol to make 100 ml.

Standard nicotinic acid solutions (20 and 10 μg./ml.)—Dilute the stock nicotinic acid solution to 1 in 50 and 1 in 100 with 25 per cent. alcohol. These standards should be prepared every month or so. The lower proportion of alcohol as compared with the stock solution is to avoid concentration by evaporation of the alcohol in the more frequently opened standard solutions.

Lloyd's reagent—Hydrated aluminium silicate obtained from Eli Lilly & Co. Each batch should be checked for adsorption and elution of nicotinic acid.

p-*Aminoacetophenone* ("*PAAP*")—Commercial samples may exhibit a strong yellow colour in acid solution and in that event should be heated in aqueous solution with "charcoal for adsorption" and recrystallised from water and tested to see that they give low reagent blanks.

PREPARATION OF EXTRACT—

Mix 1 g. of sample (in No. 30 or 40 powder for barley or malt, and containing up to 150 μg. of nicotinic acid) with 5 ml. of concentrated hydrochloric acid and water to make 15 ml. and place in a boiling water bath for 40 minutes. Cool to room temperature and centrifuge (A). Decant the supernatant liquid, add to it 2·5 g. of Lloyd's reagent, shake for 1 minute and centrifuge (B). Reject the supernatant liquid B. Wash the residue from A with 10 ml. of 0·2 *N* sulphuric acid and centrifuge. Add the supernatant liquid from this to the residue from B. Shake this mixture for 1 minute, centrifuge and reject the supernatant liquid. Add to the residue 10 ml. of 0·1 *N* sodium hydroxide (more if necessary) to make alkaline to phenolphthalein. Shake vigorously for 1 minute and make up to 16·5 ml. (≡ 15 ml. of original acid extract). Foam can be dispersed by a drop of alcohol before adjusting to volume. Centrifuge, decant and reject the residue. To the supernatant liquid add very finely powdered lead nitrate (AnalaR) to bring the *p*H to between 8·0 and 9·0, using thymol blue as external indicator and keeping the liquid well stirred to avoid adding an excess. Centrifuge and add to the supernatant liquid one drop of phenolphthalein indicator. If a pink colour is not obtained, add sufficient solid tribasic sodium phosphate to produce a pink colour and centrifuge again. Take 5 ml. of the supernatant liquid and add 1 drop of 20 per cent. phosphoric acid and then tribasic sodium phosphate to bring the *p*H to 6·5 ± 0·3, using bromothymol blue as external indicator. Make up to 25 ml. (= "final extract").

DEVELOPMENT OF COLOUR—

Prepare the following mixtures:—

	Total reagent blank TRB	"Unknown"		"Unknown" with nicotinic acid	
		U	U	UNA	U2NA
Final extract, ml.	—	5	5	5	5
Water, ml.	6	1	1	—	—
Nicotinic acid standard solution, 20 μg./ml. in 25% EtOH, ml...	—	—	—	—	1
Nicotinic acid standard solution, 10 μg./ml. in 25% EtOH, ml.	—	—	—	1	—
CNBr reagent, ml.	2	2	2	2	2

Heat in a water-bath at 56° to 58° C. for 4 minutes, cool to 20° C. for 5 minutes and then add to each:

PAAP, 10% solution in 96% EtOH, ml.	1	1	1	1	1
HCl, 6% w/v, ml.	1	1	1	1	1

Keep the mixtures in the dark, and 5 minutes after adding the PAAP and hydrochloric acid measure the optical density of U, UNA and U2NA against TRB in the Spekker absorptiometer as described below.

Prepare an *acid alcohol blank* (AAB) by mixing 20 ml. of alcohol, 20 ml. of 6 per cent. hydrochloric acid, 14·9 ml. of 0·1 *N* sodium hydroxide and water to make up 100 ml.

Prepare a *solution blank* (SB) by mixing 4 ml. of final extract with 4 ml. of the acid alcohol blank. Measure its colour against water mixed with an equal volume of the acid alcohol blank.

OPTICAL MEASUREMENTS—

Measure the characteristic yellow colour by means of a Spekker absorptiometer, using Chance's No. 7 and H503 filters before each photo-cell. The latter filter should be nearest to the photo-cell, which it protects against the disturbing effect of heat rays. Take four readings rapidly at the required time on each solution and use their mean for calculating results. To avoid errors arising from gradual variations in the response of the photo-cell, check the latter two or three times a day against a Chance's No. 6 filter wedged against a water-cell in focus on the side nearest to the photo-cell. Set the zero (with No. 7 and H503 filters immediately in front of each photo-cell as usual). Bring into focus a water-cell with no filter adjoining it and take four readings. Their mean may vary from 0·25 to 0·28 with different photo-cells and filters, but should not vary more than 0·002 from the average with a given photo-cell and filter. Undue exposure to light and heat (*e.g.*, by omitting to use the H503 filter) may cause variations of 0·01 to 0·02 or 0·025, leading to serious errors in results.

CALCULATION—

Let the mean colour of U against TRB be U/TRB, and similarly those of UNA and U2NA be UNA/TRB and U2NA/TRB. Let the colour of the solution blank against the diluted acid alcohol blank be SB/AAB.

Then the nicotinic acid content of the food is

$$\frac{\mathrm{U/TRB} - \mathrm{SB/AAB}}{\mathrm{UNA/TRB} - \mathrm{U/TRB}} \times \frac{15}{1} \times \frac{25}{5} \times \frac{10}{5}\ \mu\mathrm{g./g.}$$

or

$$\frac{\mathrm{U/TRB} - \mathrm{SB/AAB}}{\mathrm{U2NA/TRB} - \mathrm{U/TRB}} \times \frac{15}{1} \times \frac{25}{5} \times \frac{20}{5}\ \mu\mathrm{g./g.}$$

MICROBIOLOGICAL METHOD

The original method of assay with *Lactobacillus arabinosus* 17/5 as test organism[15] was later modified.[16,17,18] The method of the Microbiological Panel of the Vitamin Sub-Committee of the Society[18] is essentially that used in the present communication.

The method of computation of the results has given rise to some discussion (*cf.* Finney,[19] Wood[20]). The present results were calculated in the earlier stages by reference to the equation for the standard curve, which was calculated by the method of least squares. Later, results were calculated by comparison of the slopes of the calculated sample and standard curves. This method is to be preferred; but it should be pointed out that if the assay is valid, and sample and standard "blanks" are coincident, or very nearly so, then results calculated by either method will agree closely.

The accuracy of the method depends not only on the operator, but to some extent on inevitable slight variations of the conditions under which the living organisms grow. An arbitrary allowance of ± 10 per cent. of the mean has been considered reasonable; but experience shows that this margin may often be considerably reduced. The limits of accuracy were calculated in some instances by the method of Finney,[19] but this elegant method is somewhat lengthy when large numbers of assays are being performed.

Whatever the method of computation adopted in any particular case, no results are included which were derived from assays of doubtful validity. Thus, the microbiological results given in Table III are in every instance means of at least four valid assays, each calculated at three or four levels of sample dosage, the standard error being well within ± 10 per cent., and often within ± 5 per cent.

RESULTS

RATE OF FORMATION AND FADING OF COLOUR—This depends on the aromatic amine used and also on the material and the medium in which it is being examined. Fig. 1 summarises results we have obtained in a large number of assays on cereals and cereal products. For convenience in comparison the optical density at different intervals after mixing is plotted as the percentage of the maximum value obtained. It will be seen that the colour develops slowly with metol, more rapidly with aniline or alcoholic *p*-aminoacetophenone (at least 80 per cent. of alcohol in the reaction mixture), or procaine in 10 per cent. hydrochloric acid, and still more rapidly with aqueous *p*-aminoacetophenone (not more than 10 per cent. of alcohol in the reaction mixture). In general, the more rapidly the colour develops the more rapidly it fades. The most rapid fading is with aqueous *p*-aminoacetophenone. Unfortunately, high concentrations of alcohol cause precipitation with malt extracts. We have had to use aqueous *p*-aminoacetophenone and, as can be seen from Fig. 1, this means that the 5-minutes' interval between mixing with reagent and taking readings must be controlled to within half a minute to reduce errors to 1 per cent. Even with alcoholic *p*-aminoacetophenone appreciable errors may be introduced if the readings are taken at different times during the first 15 minutes after addition of the reagent, as has been recommended, and in our experience timing of the readings is also an advantage here. Since the colours develop much more rapidly than they fade (*cf.*[21,22,23]) readings should be taken *after* the maximum has been reached. This is ensured by an interval of 5 minutes when aqueous

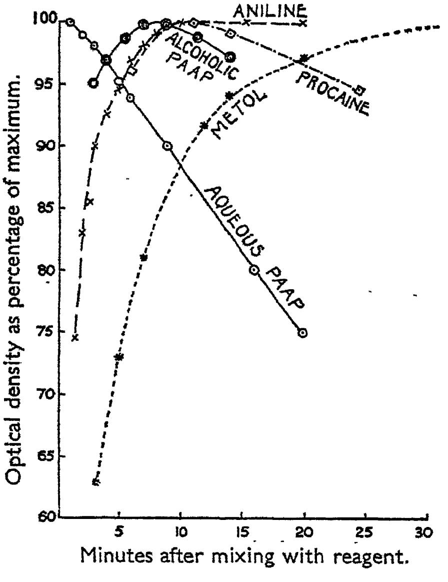


Fig. 1. Rates of development and fading of colour given by nicotinic acid with different aromatic amines used in chemical assays on cereal foods.

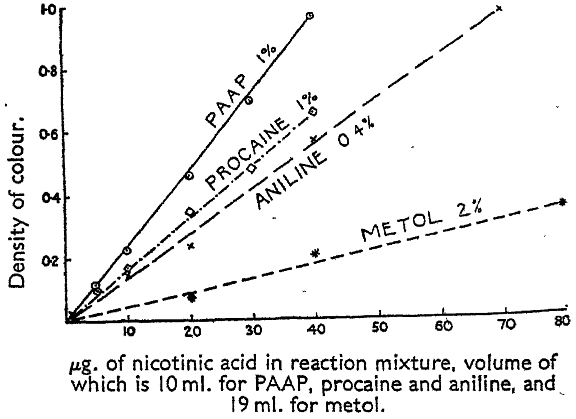


Fig. 2. Calibration curves for nicotinic acid, using *p*-aminoacetophenone, procaine, aniline or metol as aromatic amine, the percentage of each in the reaction mixture being stated.

p-aminoacetophenone is used, 10 minutes with alcoholic *p*-aminoacetophenone or procaine, 10 to 15 minutes with aniline and 40 to 60 minutes with metol. American workers[21,22] obtained a maximum colour with aniline in 6 minutes, but this might be due to their using higher temperatures. Our results were obtained at 20° to 25° C. Over this range we found no significant effect of temperature on the rate of development or of fading of colour. None of the extracts contained any nicotinamide, with which the colour develops and fades more rapidly than with nicotinic acid. Although the aniline curve in Fig. 1 reaches its maximum in about 10 minutes, statistically the results were not significantly below 100 % after 8 min.

CALIBRATION CURVES—Fig. 2 summarises our results in constructing calibration curves for nicotinic acid, using different aromatic amines. The percentage of each amine in the reaction mixture is stated in the figure. For aniline and metol these percentages are similar to those used by American workers. Higher readings on a given amount of nicotinic acid can be obtained by increasing these percentages, but, with our materials, this raised difficulties in regard to solubilities. For *p*-aminoacetophenone and procaine the percentages used were almost optimal, and small variations made no appreciable differences to the results. For instance, doubling the percentage of procaine increased the colour density by only 10 per cent. Because of these differences in solubilities we employed a reaction mixture of 19 ml. for metol and of 10 ml. for the other amines. Under these conditions, the optical density produced by metol was only a fifth of that produced by *p*-aminoacetophenone, less than a third of that produced by procaine and less than a half of that produced by aniline. Moreover, the results given by metol were much more variable, the coefficients of variation of a single observation being 11·9 as compared with 5·6 for aniline, 3·8 for procaine and 2·4 for *p*-aminoacetophenone. These results were obtained from readings taken at the time of maximum intensity, which varies widely in different experiments. In practice, therefore, it is more convenient when working with aqueous *p*-aminoacetophenone or with procaine to take readings 5 or 10 minutes, respectively, after mixing. Table I shows that this does not diminish the accuracy when *p*-aminoacetophenone is used, the standard deviation of 0·50 on 22·3 being equivalent to a coefficient of variation (for each point on the curve) of 2·2. If conditions are arranged so that the optical density readings lie between 0·2 and 0·7 the error can be reduced still further.

TABLE I

CALIBRATION OF ABSORPTIOMETER WITH PURE NICOTINIC ACID (N.A.) USING *p*-AMINOACETOPHENONE

Concn. of N.A., µg. in 10 ml.	Optical density (D) at		$\frac{\text{D max.}}{\text{concn.}}$	$\frac{\text{D in 5 min.}}{\text{concn.}}$
	max	5 mins.	× 1000	
5	0·118	0·113	23·7	22·6
10	0·226	0·213	22·6	21·4
20	0·462	0·439	23·2	22·0
30	0·697	0·663	23·2	22·1
40	0·962	0·919	24·1	23·2
		means	23·4	22·3
		standard deviations	0·57	0·50

Each curve in Fig. 2 was obtained at the optimal *p*H for the respective aromatic amine. For aniline this was that of the final extract (about 6·8). For the other amines it was more acid (about 1 for aminoacetophenone or procaine). The aniline colour is more stable but less intense at a more acid *p*H.

CHOICE OF AROMATIC AMINE—Satisfactory results were given with procaine (10 per cent. w/v in 10 per cent. w/v hydrochloric acid) and with *p*-aminoacetophenone (using not more than 10 per cent. alcohol in the reaction mixture, to avoid precipitation). Aniline is unsuitable for assaying animal extracts.[1] For cereals aniline appears satisfactory, although, like metol, it gives less intense and more variable colours under the given experimental conditions.

EFFECT OF PERMANGANATE—Wang and Kodicek,[2] using a visual method, recommended for clinical tests the use of potassium permanganate for removing interfering colours prior to development of the yellow colour given by nicotinic acid with cyanogen bromide and the aromatic amine. They did not apply the permanganate treatment to foods. We have found it to give fairly satisfactory results with unmalted barley, but obtained very variable results on applying it to malted preparations. This may be due to gradual development of haziness in the solution, to which the photoelectric method is very sensitive, so that the absorptiometer readings, when plotted against time, do not show the characteristic fall and may eventually rise considerably above the initial value. Fig. 3 illustrates such results obtained with a typical malt extract and shows that the effect may be swamped by adding excess of nicotinic acid, indicating that it is due to interfering substances. It will be seen from the figure that this action of permanganate should lead to higher results, even if these are calculated on readings taken 5 minutes after mixing. This in fact occurred. In a large number of assays on the same malt extract we found that the result could vary from 80 to 170 μg./g. according to the amount of permanganate used (see Fig. 4). The microbiological result was 102 μg./g. In practice the amount of permanganate used would not of course vary so widely as this, but it might vary sufficiently to cause considerable errors in the results. Permanganate can also cause similar increases in the absorptiometer readings obtained in the assay of nicotinic acid in milk. Fig. 5 summarises readings obtained using aminoacetophenone and procaine with permanganate. When the same samples were assayed by our method using Lloyd's reagent, no such increase in readings occurred.

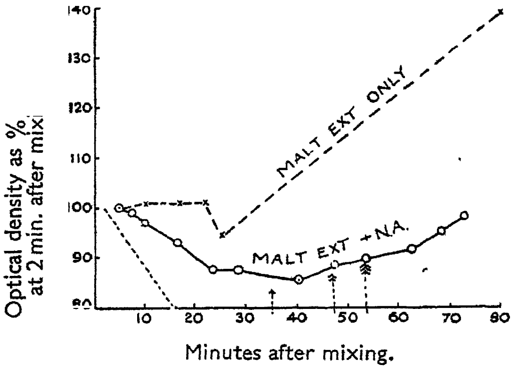


Fig. 3. Increase in density of colour given by nicotinic acid from malt extract on standing with PAAP after $KMnO_4$ had been used to decolorise interfering substances. Lower continuous curve obtained with same quantity of the malt extract but with added nicotinic acid (25 to 30 per cent. of total). Normal rate of fading in absence of $KMnO_4$ is shown by dotted curve on left, taken from Fig. 1. Arrows indicate times at which haziness was first detected visually by different observers.

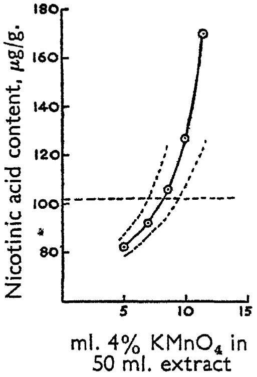


Fig. 4. Effect of amount of $KMnO_4$ used to decolorise malt extract on nicotinic acid content as found by chemical method. The broken curved lines represent twice the standard deviation. The broken horizontal line indicates the nicotinic acid content as found by microbiological assay.

Improvement of accuracy—When we first applied Wang and Kodicek's method to cereals and cereal products our chemical results averaged only 75 per cent. of the microbiological results and varied widely, as shown by the coefficient of variation of a single result sometimes exceeding 20. This was due to the use of too little permanganate and imperfect control of the amounts (see Fig. 4). We were able to improve the reproducibility of our results and bring them much closer to the microbiological results by standardising the amount of potassium permanganate used at 9 ml. of 4 per cent. solution for 50 ml. of reaction mixture, by fixing the temperature of the cooling water at 20° C. and by using Melnick's solution blank. The chemical results then averaged 109 per cent. of the microbiological results and the coefficient of variation of a single assay was reduced to 11·6. This improvement in accuracy was due mainly to control of the permanganate additions. Further improvement was sought by trying the American adsorption techniques.

Removal of interfering substances by adsorption—Various adsorbents have been employed for this purpose by American workers. Charcoal was at one time recommended. Our experience with B.D.H. "charcoal for adsorption" gave unsatisfactory results owing to incomplete adsorption of nicotinic acid. For example, at *p*H 6·4 only 55 per cent. was adsorbed in a malt extract assay. The solution blank was 19 per cent. of the total colour, no better than was obtained with permanganate. Further experiments under different conditions still showed incomplete adsorption. We then tried Lloyd's reagent, which has been widely used in America and is recommended by a Niacin Assay Committee of representative American workers. In our first experiments with this we used 1 g. for each g.

of material containing up to 150 μg. of nicotinic acid. After heating 1 g. of the sample for 30 to 40 minutes in a boiling water bath with 3 N hydrochloric acid and cooling to room temperature, the supernatant liquid (which had a *p*H below 1) was shaken for 1 minute with 1 g. of Lloyd's reagent. The adsorbed nicotinic acid was eluted with 0·1 N sodium hydroxide and the resulting solution clarified with solid lead nitrate and trisodium phosphate as recommended by the American workers. Our results showed improved reproducibility (the coefficient of variation of a single assay having dropped to 6·5) but averaged only 91 per cent. of the microbiological results. By increasing the amount of Lloyd's reagent from 1 g. to 2·5 g. and introducing other slight modifications in technique we brought the average chemical results up to 101 per cent. of the average microbiological results. The coefficient of variation was reduced to below 6 and the recovery of nicotinic acid added to the original material was 98 per cent. No further increase in the chemical results was produced by raising the amount of Lloyd's reagent to 5 g. per gram. Decolorisation with zinc hydroxide in addition to lead hydroxide has been recommended by Swaminathan.[24] Our experiments with his technique have not given any better results than those obtained by our usual method employing Lloyd's reagent and lead hydroxide.

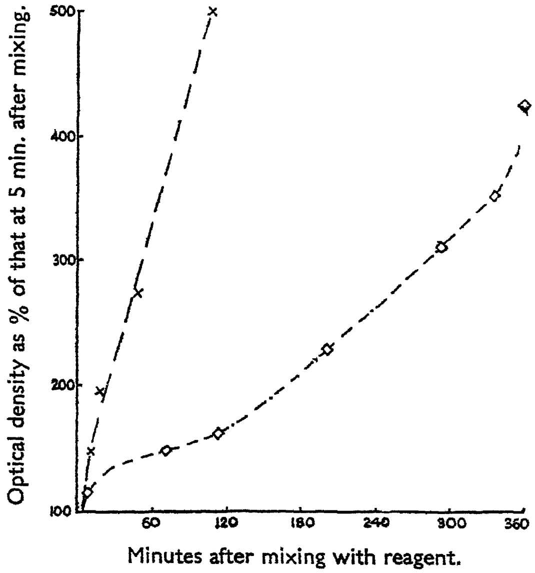


Fig. 5. Increase in density of colour given by nicotinic acid from milk on standing with reagent, when $KMnO_4$ had been used to decolorise interfering substances. Results obtained with PAAP indicated thus: × —— ×; results with procain thus: ⊡ —.— ⊡.

Correction for interfering substances—Interference with the colour given by the reaction between nicotinic acid and the reagent may be caused by:

(*a*) the colour of the reagent,
(*b*) the colour of the test solution,
(*c*) changes in colour produced by reaction between the reagent and non-specific substances in the test solution.

Correction for (*a*) is usually made by means of a reagent blank containing the amine and cyanogen bromide at the final *p*H. When *p*-aminoacetophenone is used a high reagent blank may be obtained, due mainly to the amine. This has led some workers[25] to substitute an amine blank for the total reagent blank, but in our experience this is not entirely satisfactory. The optical density of the total reagent blank may be 20 to 40 per cent. of the total optical density obtained in the assay.

Correction for (*b*) is usually made by means of the test solution at the final *p*H but containing no reagent. This test blank has also been termed "colour blank" or "solution blank" by different workers. The solution blank may vary widely with different foods and decolorising procedures and can have a colour greater than that produced by the nicotinic acid. Much work has therefore been done with the object of reducing its colour. Its magnitude in relation to the total colour obtained in the assay has in fact been suggested for measuring the efficiency of decolorising procedures. When, however, we applied this criterion to compare the value of permanganate with Lloyd's reagent the differences were not very significant, as can be seen from Table II, which is based on over 200 assays on numerous samples. On the whole, Lloyd's reagent gave lower blanks. With wheat flour and meat extract the average blanks were considerably lower, but only two samples of each were examined. With cereals and other foods, of which more samples were examined, the differences were less evident.

TABLE II

EFFECT OF DECOLORISING PROCEDURE ON INTERFERING SUBSTANCES

	Optical density of solution blank as percentage of total optical density in assay after decolorisation with:	
	$KMnO_4$	Lloyd's reagent and $Pb(OH)_2$
Wheat flour	38	27
Barley	13	13
Malted barley	15	16
Malt extract	18	15
Yeast	22	21
Milk, dried	64	61
Meat extract	40	16
Maize (yellow corn meal)	31	30

Correction for (*c*) is a more complicated procedure involving the use of further blanks to determine the effect of amine and of cyanogen bromide separately on the test solution. This procedure has been carefully studied by Friedemann and his colleagues in America.[26] The application of Friedemann's methods to our materials has raised the question of the effect of germination, which will be considered in detail elsewhere. Here it will suffice to say that by using the reagent and solution blanks as described on p. 329 we have obtained good agreement with microbiological results on a wide variety of foods. Certain discrepancies (especially in low extraction flours) cannot be satisfactorily explained as due to different blanks, and other sources of error are being considered.

RECOVERY EXPERIMENTS WITH ADDED NICOTINIC ACID—These have been used by various workers to test the validity of Beer's law, the nicotinic acid being added after the decolorising treatment. Under these conditions we have obtained fairly complete recovery of added nicotinic acid but did not find the results of much value in correcting for interfering substances. On the other hand, we think it essential to test the behaviour of Lloyd's reagent with nicotinic acid under the experimental conditions, preferably by adding a known amount of nicotinic acid to the foodstuff being examined. Under these conditions also we have been able to obtain complete recovery.

COMPARISON OF CHEMICAL AND MICROBIOLOGICAL RESULTS—These are summarised in Table III (p. 335).

Taking into consideration the respective errors of the two methods, there was a highly significant difference with the two low extraction wheat flours, yellow corn meal and yeast extract. The chemical results were considerably higher, except on the yeast extract, which caused difficulties often encountered with yeast preparations. In the collaborative study by the American Niacin Assay Committee under the chairmanship of Dr. D. Melnick it was also found that chemical methods gave considerably higher results than microbiological methods on low extraction wheat flour, whilst satisfactory agreement was obtained between the two methods on whole wheat flour. It was suggested that this might be due to the presence of a nicotinic acid precursor becoming available to the micro-organism only after hydrolysis. In our work the question of this precursor is being studied in relation to the precursor in cereals which yields nicotinic acid on germination, and will be reported upon later. Results so far obtained indicate that the two types of precursors are probably different. The first two samples in Table III are samples No. 1 and No. 8 in the above American collaborative study. Our chemical and microbiological results on these are in excellent agreement with the average chemical and microbiological results obtained by leading American workers. The sample of maize (yellow corn meal) in Table III was supplied by Dr. Elvehjem, whose microbiological result of 19·5 µg./g. was in good agreement with our microbiological result of 22 µg./g. Our higher result, using the final chemical method, was not related to a specially high solution blank, and some other explanation must be sought. Several of the other samples in Table III have also been examined by other workers, whose results confirmed ours, and we think that, apart from the low-extraction flours, maize and yeast, we have established satisfactory agreement between chemical and microbiological results in the range of samples examined.

TABLE III

NICOTINIC ACID CONTENT OF CEREALS AND OTHER FOODS

	Nicotinic acid µg./g. by	
	chemical assay	microbiological assay
Whole wheat flour	62	61
Whole wheat bread	68	62
Wheat germ	134	129
Flour, National	27	14
„ white	47	20
Barley	109	111
Malted barley	126	127
Malt extract, commercial sample	102	95
„ „ „ „	99	96
„ „ „ „	90	96
Malt extract, dried	111	110
Yeast, dried	276	290
„ „	346	320
„ „	290	290
„ „	332	331
Yeast extract	478	537
Dried meat	162	135
Meat extract	1027	1082
Yellow corn (maize) meal	43	22
Malt and yeast food	99	79
„ „ „ „	113	116

Notes:

The nicotinic acid content is expressed as µg. per g. of the food as received, and each result is the mean of several assays. The following standard deviations of these means showed a highly significant difference between chemical and microbiological results on the National flour (chem. 2·2, microbiol. 0·2), white flour (chem. 3·6, microbiol. 0·4), yellow corn meal (chem. 3·0, microbiol. 0·4) and yeast extract (chem. 15·0, microbiol. 5·3) and a significant difference on the first sample of malt and yeast food (chem. 6·0, microbiol. 1·7). With the remaining sixteen samples there was no significant difference.

The accuracy of the chemical method with maize, yeast, and wheat flours of less than 90 per cent. extraction was found to be considerably lower than with the other foodstuffs examined. Thus, in 19 assays on 5 samples of the above the average coefficient of variation of a single assay was 12·7, as compared with an average of 5·8 in 55 assays on the remaining 16 samples. In calculating these coefficients the only results excluded were a few obtained mainly by Kodicek's method where development of cloudiness interfered with the Spekker readings. The lower standard deviations of the means for the microbiological method were due partly to its greater accuracy and partly to more assays being made on each sample.

These findings are in general agreement with the findings of most other workers both in this country and in America. Certain American workers[27,28] have reported that microbiological methods give considerably higher results than chemical methods (using Lloyd's reagent and *p*-aminoacetophenone or metol) on wheat, barley or malted barley, but that this discrepancy disappears when the material is oxidised with hydrogen peroxide before carrying out the microbiological assays. Their results have not been confirmed by other American workers[16] and need further investigation.

SUMMARY

Difficulties have been encountered in the chemical assay of nicotinic acid in cereals. A study, using the Spekker photo-electric absorptiometer, has been made of the following factors which might affect the results.

(*a*) Choice of and method of using aromatic amine, including *p*-aminoacetophenone in alcoholic or aqueous solution, procaine, aniline and metol.

(*b*) Procedures for removing or correcting for the presence of interfering substances by decolorisation or adsorption techniques or by the use of different blanks.

(*c*) Instrumental errors.

A chemical method has been devised which has given satisfactory reproducibility and agreement with microbiological results on a wide range of cereal products. This method gave considerably higher results than the microbiological method on low-extraction flours, maize, and some yeast preparations, but the discrepancy (which has also been encountered by other workers) cannot be explained by any of the above factors. The possible effects of nicotinic acid precursors have been left for further study.

We are indebted to Dr. D. Melnick and his colleagues on the Niacin Assay Committee, to Dr. E. R. Dawson for providing collaborative samples, and to Miss Janet Horsford for technical assistance.

The chemical work undertaken by one of us (E.M.J.) in this investigation forms part of the work for a Ph.D. thesis in the University of London.

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OVALTINE RESEARCH LABORATORIES
KING'S LANGLEY, HERTS.

January, 1947

A Note on the Chapman and McFarlane Method for the Estimation of Reducing Groups in Milk Powder

BY C. H. LEA

(*Read at the Meeting of the Society on April 2nd*, 1947)

WHILE peroxide, Kreis and aldehyde values of the fat, in addition to measurement of absorption of oxygen and production of carbon dioxide by the powder, have been used with some success in confirmation of organoleptic tests for fat deterioration in full cream milk powder[1,2,3] specific chemical tests for the protein type of spoilage which leads to the production of stale and "gluey" flavours and loss of solubility in separated milk powder or in full cream powder of high moisture content have been lacking. For this reason the colorimetric method for the estimation of reducing groups in milk powder recently proposed by Chapman and McFarlane[4] is of particular interest.

The method of Chapman and McFarlane depends on the reduction of potassium ferricyanide solution by heating with the powder for 20 minutes at 70° C. and *p*H 5·0, after which the protein is precipitated with trichloroacetic acid and the ferrocyanide produced estimated colorimetrically after development with ferric chloride. The duration and temperature of heating were chosen to give a large difference between fresh and deteriorated powders, and the reaction does not proceed to completion.

Following Mirsky and Anson,[5] the authors conclude that the reducing groups responsible for the reaction of stale or heated milk powder, casein or lactalbumin are present in the protein molecule and become accessible on denaturation, and that these groups are probably mainly sulphydryl, assisted perhaps by tryptophan residues. Chapman and McFarlane therefore express their results as moles × 10^{-5} reducing groups per gram of powder, and calibrate against the SH group, using glutathione or cysteine as standard.* The values obtained did not obey Beer's law, apparently because the ferric ferrocyanide produced was not in true solution.

We have found that Beer's law is obeyed, and a straight line calibration curve obtained, provided that the coloured solutions are matched one minute after mixing instead of after 10 minutes as recommended in the original method. The occurrence of obvious flocculation in the stronger solutions on prolonged standing indicates the necessity for this modification. Using a Spekker photo-electric absorptiometer with a red filter, we obtained the same linear calibration curve passing through the origin and extending to an extinction coefficient of approximately 2·8 whether the colour was produced by known small quantities of potassium ferrocyanide or of ferrous ammonium sulphate, development of the colour in each case being with ferric chloride in the presence of potassium ferricyanide, phthalate buffer and trichloroacetic acid as in the test. Since evidence presented here and elsewhere[6] concerning the part played by sugar in causing deterioration of the protein leaves in doubt the precise nature of the groups causing reduction of the ferricyanide, it has been preferred to express the results as moles × 10^{-5} of ferricyanide reduced per gram of dry milk solids. Such values are not directly convertible to SH groups or to cysteine units since, under the conditions of the test, cysteine does not reduce exactly one equivalent of ferricyanide.

RESULTS

Preliminary tests with the method showed that reasonable variation in the quantity of powder taken for the determination did not affect the result provided that the colour developed was not too deep. Fresh spray-dried powders showed a small reducing power which increased on storage either rapidly or very slowly according to the moisture content of the powder and the storage temperature.[6,7]

NATURE OF THE REDUCING SUBSTANCES—

Mirsky and Anson[5] found SH groups and tryptophan and tyrosine residues to be mainly involved in the changes in reducing power that result from the denaturation of proteins. Chapman and McFarlane found that ferricyanide under the conditions of their test was reduced strongly by cysteine, feebly by tryptophan and not at all by tyrosine. The amount of cystine present in milk powder, however, even if all available as free cysteine, is not sufficient to account for as much as 10 per cent. of the reducing power of a badly deteriorated milk powder. Similarly, tests carried out with tryptophan and tyrosine in the proportions reputed to be present in separated milk powder show that these acids, even if entirely exposed in the protein, could not account for more than about 5 per cent. and 0·3 per cent. of the reducing power of fresh spray-dried powder, or about 0·2 per cent. and 0·0 per cent. of the reducing power of a badly deteriorated powder. Lactose showed no reducing power. Glucose also showed no reducing power, but after treatment with *N* sodium hydroxide at 40° C. for 5 minutes developed a pronounced brown colour and a reducing power of 6·3 moles × 10^{-5} of ferricyanide per gram.

Dialysis of a fresh milk powder of high (7·6 per cent.) moisture content showed that of its total reducing power of 0·9 moles × 10^{-5} ferricyanide per gram, about 0·6 was dialysable, while 0·3 remained associated with the protein. After storage at 37° C. for 6½ months the powder, which was now brown in colour and of very poor solubility, showed a total reducing value of 22·1 per gram of dry solids, of which about 9 units were dialysable and 13·1 undialysable. Approximately one-fifth of the undialysable fraction was still soluble, and there had been no increase in the proportion of dialysable nitrogen during storage. These observations suggested that a non-dialysable protein - carbohydrate complex or its decomposition products was mainly responsible for the increased reducing power of the deteriorated milk powder, assisted in some degree by dialysable, non-nitrogenous reducing material, presumably degradation products of lactose.

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* In a private communication received since completion of the present work, Professor R. A. Chapman states his belief that the substances responsible for the increase in reducing capacity in milk powders are either a complex formed between an amino group and a carbohydrate or decomposition products of such a complex.

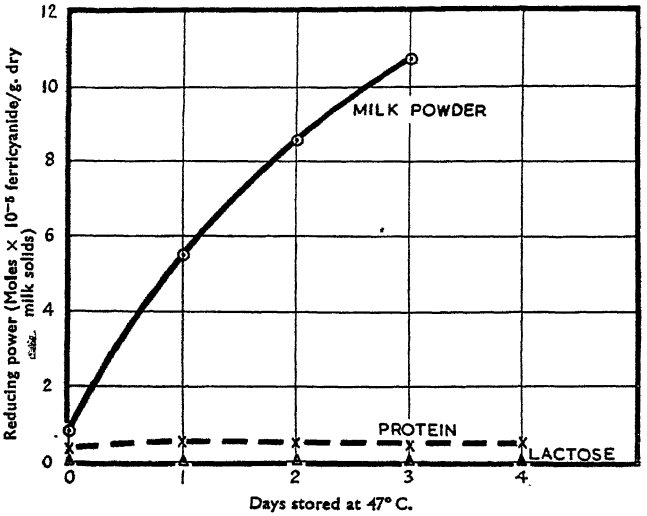

Fig. 1. Development of reducing power by (*a*) separated milk powder, (*b*) milk protein (dialysed separated milk powder) and (*c*) lactose, during storage at 47° C. and 55 % relative humidity.

STORAGE OF THE PROTEIN AND CARBOHYDRATE FRACTIONS OF MILK POWDER—

A quantity of fresh, spray-dried separated-milk powder was reconstituted, dialysed in cellophane against distilled water for 3 days at 0° C., and freeze-dried in high vacuum at a low temperature. Separate samples of this material, of pure α-lactose hydrate and of the control (undialysed) milk powder were stored in thin layers at 47° C. over sulphuric acid giving a relative humidity of 55 per cent. and examined daily for reducing power and moisture content. The results, calculated in all cases to moles × 10^{-5} ferricyanide per gram of dry, separated-milk solids are given in Fig. 1. The residual "protein" fraction and the lactose, when stored separately, showed practically no increase in reducing power under conditions which caused rapid deterioration of the separated-milk powder.

In a second experiment fresh, separated milk was dialysed at 0° C. for 7 days and the residual "protein" admixed with various sugars, frozen rapidly, freeze-dried, ground and stored at 37° C. and 55 per cent. relative humidity for 4 months. The compositions and reducing powers of the products are given in Table I. The *p*H of all samples lay between 7·00 and 7·05.

TABLE I

EFFECT OF VARIOUS SUGARS ON THE DEVELOPMENT OF REDUCING POWER

Sample	Sugar added (g./100 g. dry protein)	Reducing power (moles × 10^{-5}/g. protein)		Loss of free amino N during storage (mg./g. total N)*
		Initial	Increase during storage	
Control ..	—	0·6	0·2	0
G	1·9 glucose	0·5	16·1	7
L	144 lactose	0·4	31·0	33
S	144 sucrose	0·4	0·3	0
GL	1·9 glucose 144 lactose	0·5	34·4	35
GS	1·9 glucose 144 sucrose	0·4	13·4	

* Initial value = 60.

It appears from these results that the ferricyanide reducing power of milk powder is an index of the formation and degradation of a protein - sugar complex, rather than of simple denaturation of the protein, that glucose is much more active than lactose in undergoing such reaction with protein, and that sucrose is inert. Since the protein - sugar reaction is now known to be a major factor in the causation of non-fatty deterioration in milk powder of high moisture content,[7] and probably in other foods such as dried egg,[8] the Chapman and McFarlane test, which is easily carried out as a routine procedure, appears to have definite possibilities for the control of quality. No single chemical test, however, can be expected to correlate accurately with flavour over a wide range of moisture contents, storage temperatures and storage atmospheres because at least two or three separate mechanisms appear to be involved in the development of "off" flavour under widely different storage conditions.

SUMMARY

The increase in the reducing power towards potassium ferricyanide which develops during the storage of milk powder, particularly at high moisture contents and high storage temperatures, has been shown to be due to the formation and degradation of a protein-reducing sugar complex.

Technical assistance in this work was given by Mr. D. N. Rhodes. The work was carried out as part of the programme of the Food Investigation Board and is published by permission of the Department of Scientific and Industrial Research, Low Temperature Station for Research in Biochemistry and Biophysics, University of Cambridge and Department of Scientific and Industrial Research.

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LOW TEMPERATURE RESEARCH STATION
CAMBRIDGE

February, 1947

DISCUSSION

Mr. E. B. ANDERSON said that work carried out in their laboratory confirmed Dr. Lea as to the formation of reducing substances in the heat treatment of milk, and the interaction of reducing sugars with amino groups. The new modification of the Chapman and McFarlane method would be of interest, as they had not had much success with the method. He would be interested to know the level of reducing substances as shown by this test in spray-dried powder from the high preheat treatment and also the level for roller powder.

Mr. A. L. BACHARACH asked if there was any indication that fat in a full-cream milk powder was able to reduce protein deterioration, apart from its effect as a neutral diluent. In his view, available data pointed to the superiority of spray powder over roller powder in resistance to protein deterioration, though the reverse was true of fat spoilage. The problem of carbohydrate and protein combination, presumably primarily a reaction between the aldehyde group of a sugar and the amino group of an amino acid, and of the subsequent breakdown of intermediate products, deserved, in his view, fundamental study in which presumably modern physical methods could play a valuable part in helping to elucidate changes of configuration in the protein molecule. He trusted that Dr. Lea and his colleagues would themselves be undertaking such studies or would at any rate encourage others to do so.

Dr. MAGNUS PYKE said he was very interested in the subject and anxious to learn what was the chemistry of the reactions involved and to what extent they are related to the biological value of the protein. Any differences between the behaviour of vegetable and animal proteins on storage are of great interest and practical importance.

Mr. E. BARTON MANN said he welcomed the work of Dr. Lea in so far as his method might assess the degradation of dried skimmed milk. Fraps, Meinke, Reiser and Sherwood (*Bull. Texas Agric. Exper. Station*, 1943, **637**, 23) had indicated that some animal proteins, including those of dried skimmed milk and dried buttermilk, may have "a high consuming power for carotene." In feeding tests on poultry it is no uncommon thing to find this depressant action of animal feeding stuffs, in respect to both carotene and vitamin A. If there is a linkage between the lactose and the protein of milk, it is obvious that the denatured protein, as it cannot be utilised by the organism, would form food for attack by micro-organisms, most probably of the Gram-positive anaerobic type. Preponderance of these gives rise to intestinal putrefaction and mild toxicity and might perhaps interfere with vitamin and provitamin assimilation. As lactose enjoys the peculiar property of not being entirely absorbed until it reaches the end of the gut, if it is rendered non-effective by combination with protein, then there is always the possibility that nutriment may be withheld from lactic acid bacteria—the natural enemies of Gram-positive putrefactive anaerobes. Dr. Lea has recorded the lowered biological value of dried skimmed milk which by reason of age or other conditions has shown a higher lactose-protein complex than originally. Had he any reason to believe that this lowered value may be coupled with excessive bacterial action in the intestines on the lines suggested?

Dr. H. Liebmann asked if the amino acid - sugar reactions are catalysed by heavy metals.

Mr. H. W. Hartley said it had been found that in "sugar - dried egg" the sucrose exerts a protective influence on the protein, whereas in Dr. Lea's experiments, although sucrose had no adverse effect, no advantageous effects were apparent.

Dr. Lea, in reply to Mr. Anderson, said that he had not as yet specifically compared the level of reducing substances in spray-dried powders from low and high pre-heated milk. The level in roller powder was very much higher than in fresh spray powder, presumably as a result of the more severe heat treatment during drying.

In reply to Mr. Bacharach, he was not aware of any stabilising effect of the fat present in full-cream powders on protein deterioration, although there might very well be one. Separated milk powder, as a less valuable commodity, was often of higher initial moisture content (even on the fat-free basis) and was liable to be less carefully treated before, during and after drying than the full-cream product. A more fundamental attack on the reaction between pure proteins and sugars was being undertaken.

In reply to Dr. Magnus Pyke, he said that since lysine was the amino acid most concerned in the loss of biological value, the protein - sugar reaction was of particular interest with regard to the lysine-rich animal proteins that normally supplemented the cereal proteins in a mixed diet. The points raised by Mr. Barton Mann formed interesting subject-matter for speculation, but he was, at present, unable to carry them any further.

The simple amino acid - sugar reaction in the test tube is known to be catalysed by traces of heavy metals, and evidence is available that this is true also for deterioration of this type in certain dried vegetables.

In reply to Mr. Hartley, Dr. Lea stated that, although sucrose did not delay the primary reaction between reducing sugar and protein amino groups in milk powder, it did greatly delay the secondary change which resulted in loss of solubility of the protein. In this respect it would probably be found that dried milk and dried egg were similar.

The Photometric Determination of Dissolved Oxygen in Condensates and Feed Waters by means of the Starch-Iodide Complex

By S. BAIRSTOW, J. FRANCIS, and G. H. WYATT

The increasing use of boiler plant having working pressures over 250 lb. per sq. in. has made the control of the dissolved oxygen content of the feed water of great importance. The concentration of dissolved oxygen that can be allowed with safety decreases rapidly with rise in pressure above this value, and it is generally accepted that the amounts should not exceed 0·03 ml. per litre at 400 lb. per sq. in. and 0·01 ml. per litre at 600 lb. per sq. in.[1]; at pressures of 1000 lb. per sq. in. and over, dissolved oxygen cannot be tolerated and must be removed, for example, by sodium sulphite. Since it is necessary to be able not only to show that the oxygen concentration is kept below the appropriate maximum value, but also to detect small differences due to leakages of air into the system before and after adjustment of the plant, it is desirable that the method of measurement used should be capable of determining amounts of dissolved oxygen considerably less than the maximum values specified. The classical method for this determination was devised by Winkler[2] and involves oxidation by dissolved oxygen of manganous hydroxide precipitated in the solution; the mangano-manganic hydroxide (Mn_3O_4) so formed[3] reacts with potassium iodide to give free iodine which is determined volumetrically, with starch as indicator.

The Winkler method is subject to error because: (*a*) the end-point of the titration is indefinite; 0·01 ml. of dissolved oxygen per litre in a 250-ml. sample liberates iodine equivalent to only 0·045 ml. of 0·01 *N* thiosulphate solution, (*b*) the colour of the starch-iodide complex disappears while some iodine is still present[4]; at ordinary temperatures this undetected iodine is equivalent to about 0·01 ml. of dissolved oxygen per litre, (*c*) no allowance is made for interference by substances other than dissolved oxygen. The method is suitable for the examination of natural waters, in which Winkler was interested, but for modern feed water supplies a more accurate process is necessary. Modifications have been described in the literature in which various ways of determining the liberated iodine have been proposed, interference by substances other than dissolved oxygen has been measured and the sampling apparatus has been improved. The present paper describes (1) the development of an accurate method for the determination of the free iodine by measurement of the intensity of the blue starch-iodide complex with the Spekker photo-electric absorptiometer, and (2) a convenient form of sampling apparatus in which may be carried out, without atmospheric contamination, the reactions liberating iodine, including those due to interfering substances. The complete process has been in use at this laboratory for about six months, during which period over four hundred routine determinations have been made.

Other methods—Consideration was given to the "dead-stop" method of titration first described by Foulk and Bawden[5] in which the end-point of the iodine - thiosulphate titration is detected accurately by electrical indication of the polarisation, by the thiosulphate, of platinum electrodes immersed in the solution. The procedure was applied to the determination of dissolved oxygen by Hewson and Rees[6] who did not, however, estimate interfering substances. This method involves a delicate titration and it was felt that elimination of this operation is highly desirable if routine determinations are to be made by hitherto untrained persons. This could be achieved by the application of colorimetry and an examination was first made of the proposal of W. Francis,[7] whereby the oxidation product of the precipitated manganous hydroxide is treated with hydrochloric acid to form free chlorine, which is determined by the well known colour reaction with *o*-tolidine.

This reaction appears *a priori* to be capable of measuring quantities of oxygen that would remain undetected by the Winkler titration, and it was found that a solution containing free chlorine equivalent to 0·002 ml. of dissolved oxygen per litre gave with *o*-tolidine a colour readily distinguishable from a blank. Pairs of samples of water were taken simultaneously from the same source and the dissolved oxygen was determined by the *o*-tolidine and Winkler methods; the results agreed within the limits of accuracy of the latter process. After the method had been in routine use for a few months, a decrease in the values found for the dissolved oxygen concentrations was observed. The measured values continued to diminish until finally no oxygen could be detected. During this period alterations had been made to the feed water system with a view to decreasing the oxygen content of the water, but its complete elimination appeared to be highly improbable. Comparison of the *o*-tolidine and Winkler methods was, therefore, repeated, and it was found that, although no free chlorine was detected by the former, the Winkler titration indicated the presence of 0·03 to 0·05 ml. of dissolved oxygen per litre. The oxygen contents of samples of feed water were artificially increased by introducing known amounts of water saturated with air, and it was found that *o*-tolidine first indicated the presence of free chlorine when the dissolved oxygen content of the sample, as determined by the Winkler method, was 0·07 to 0·08 ml. per litre. Further investigations were not made, but it is concluded that free chlorine was removed from solution immediately on liberation, probably by minute traces of organic matter in the feed water. A slight contamination of the condensates by the cooling water occurs continuously and it is thought to be significant that concordant results were obtained by the two methods during winter months, while the discrepancy was found in the Spring, when algal growths increase.

Starch-iodide methods—The *o*-tolidine reaction was abandoned and attention was directed to the measurement of the intensity of the blue colour of the starch-iodide complex formed in the same manner as in the Winkler method. Verbestel[8] refers to the use of the colour of the iodine solution for the determination of the dissolved oxygen content of water, and standard colour discs* may be obtained for this estimation. The change in depth of colour corresponding to 0·01 ml. of oxygen per litre is, however, small and the disc for feed waters contains only nine tinted glasses for the range of concentrations from 0·05 to 1·0 ml. per litre. Katayama[9] stated in 1907 that the concentration of starch-iodide is proportional to the iodine concentration if the starch concentration is kept constant, but he did not point out the possible analytical application of his discovery. In 1926 von Fellenberg[10] reported that the intensity of the colour of the starch-iodide complex is not always proportional to the iodine concentration and that the reaction is of little value for the determination of iodine if salts are present; the latter contention was confirmed by Woodard.[11] On the other hand, Turner[12] found that the method is satisfactory for dilute solutions of iodine in absence of salts, but his paper has been adversely criticised.[13] A careful study of the reaction was made by Müller and McKenna,[14] from whose paper the extinction curve shown in Fig. 1 has been taken. At first no colour is produced; a range of iodine concentrations then follows over which the extinction curve is not straight, and finally the colour intensity is linearly related to the iodine concentration. Müller and McKenna point out that for the linear part of the curve the extinction E is related to the iodine concentration c by the relation $E = k(c - c_0)$ and state that knowledge of c_0 allows calculation of the quantity of iodine that must be added to test solutions to ensure that only the linear part of the curve is used: it is clear from Fig. 1 that an iodine concentration c_1 is required. As early as 1887 Mylius[15] had shown that the blue colour is not obtained unless an iodide is present: it has since been stated[9,16] that the depth of colour is related to the iodide concentration in a complex manner, the iodide having a markedly greater effect at lower concentrations. Recently, however, it has been considered that the effect is due to a change in the electrical charge on the particles and it has been shown that salts other than iodides behave in a similar manner (for a full account of this and other work on the starch-iodide complex, with references prior to 1930, see Barger[17]; a later review is given by Meyer[18]). Results obtained during the present investigations, however, show that former emphasis on the effect of salts was too great. Biochemists have claimed successful applications of photo-electric adaptations of the reaction to the determination of iodine in blood[12,19] and protein-bound serum[20] and of chloride, iodate and iodide in protein-free solutions.[21] Most of these applications isolate iodine by distillation, oxidise it to iodate and then liberate iodine by the iodate - iodide reaction; by a similar reaction bromate in treated flour has been determined.[22] Simerl and Browning[23] have examined the colours produced by various kinds of starch and a number of starch derivatives with a view to the determination of starch in paper.

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* For use with the B.D.H. Lovibond Nessleriser.

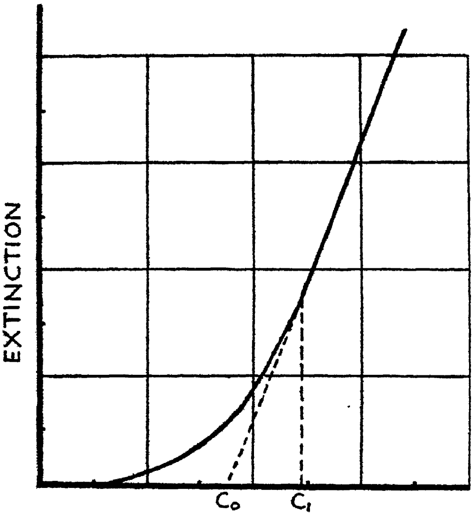

Fig. 1. Extinction curve.[14]

It is now recognised[24] that water often contains substances other than dissolved oxygen which may either liberate iodine from potassium iodide or absorb iodine, and it is therefore essential in the accurate determination of dissolved oxygen to include an "interference sample," in which is measured the effect of the interfering substances; the result obtained with the ordinary sample may then be corrected.

Experimental

Starch concentration—Preliminary experiments showed that it would probably be possible to determine up to 0·10 ml. of dissolved oxygen per litre of water by measuring the intensity of the blue colour of the starch-iodide complex by means of the Spekker photoelectric absorptiometer, using a 4-cm. cell in conjunction with a tungsten filament lamp, heat filters H503 and spectrum red filters H608. All the investigations described below were made with this equipment, but final calibration curves were drawn both for the tungsten filament and mercury vapour lamps. A 3-litre sample of feed water was collected under air-free conditions and 17·5 ml. of each of the three Winkler reagents alkaline iodide, sulphuric acid and manganous sulphate were added in that order. In these circumstances the small quantity of oxygen dissolved in the feed water does not react, since the formation of manganous hydroxide is prevented. (This solution is called the *blank solution* in the following account). Additions of standard iodine solution (approximately 0·001 *N*) were made to 250-ml. portions of the blank solution to give iodine contents equivalent to 0·01, 0·02 . . . . 0·10 ml. of oxygen per litre. The solutions were cooled to 15° C. and 5 ml. of 0·1 per cent. starch solution were added to each; after mixing and leaving to stand for 5 minutes, the absorptions were measured. It was found that insufficient starch had been added for the detection of more than about half the desired concentrations of oxygen. Accordingly, a family of curves, shown in Fig. 2, was constructed in a similar manner using starch solutions of various concentrations.[*] Points corresponding to 1·0, 1·5 and 2·0 per cent. starch solutions lie too close together to show clearly, so that curve *F* has been drawn to fit all the points concerned. In all subsequent determinations and investigations 1 per cent. starch solution was used. It is interesting to note that the ratios of the concentrations of starch and iodine at which curves *A*, *B* and *C* cease to be linear correspond to approximately 3·3 per cent. of iodine in the blue complex. Most of the late-nineteenth century researches on the constitution of the "compound" of starch and iodine attempted to prepare products with a maximum iodine content, but the value now found approximates to the minimum of 3 per cent. cited by Radley.[25]

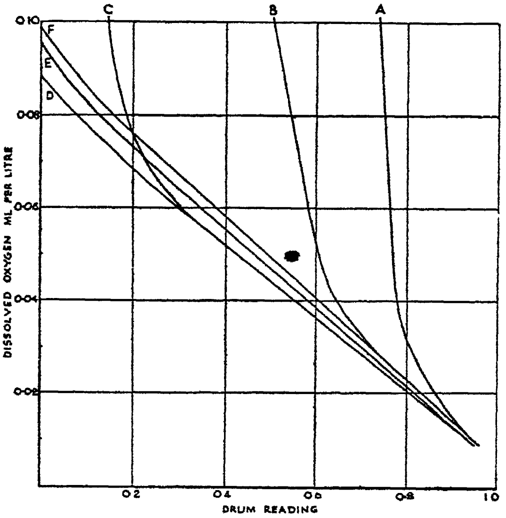

Fig. 2. Effect of starch concentration.

Curve A	..	0·05 % starch solution
„ B	..	0·1 % „ „
„ C	..	0·2 % „ „
„ D	..	0·4 % „ „
„ E	..	0·7 % „ „
„ F	..	1·0 % „ „ 1·5 % „ „ 2·0 % „ „

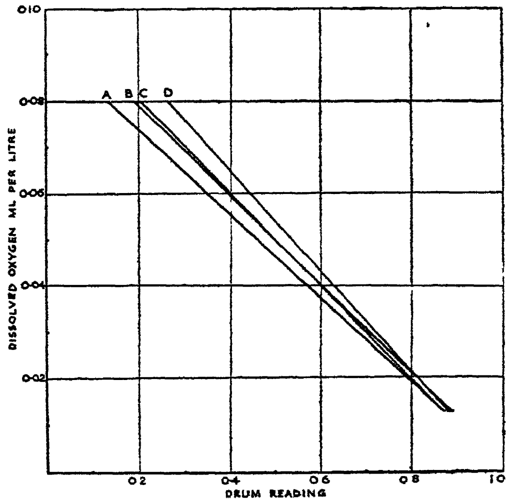

Fig. 3. Effect of different starch samples.

	Manufacturer	*Batch*
Curve A ..	a	1
	a	2
„ B ..	a	3
„ C ..	b	—
	c	—
„ D ..	d	—

Addition of starch—Throughout these investigations and in routine determinations care has been taken to use starch solutions prepared under standardised conditions, since the blue colour is usually considered to be due to a colloidal complex. However, wide differences in treatment with the same starch solution failed to produce any significant difference in the absorption of a series of blank solutions containing the same quantity of added iodine (see Table I).

The effect of using starch solution stored for various periods was also examined. The dissolved oxygen in pairs of water samples collected simultaneously was measured, using freshly prepared and stored starch solutions containing no preservative. The storage periods ranged from 1 to 8 days, but in no instance did the results with the stored starch solution differ from those with the fresh by more than 0·01 in drum reading, corresponding to 0·001 ml. of oxygen per litre—an insignificant difference. It was decided that for routine determinations the starch solution should be freshly prepared every week; since samples of feed waters are analysed on five days per week this allows a safety margin of at least three days.

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[*] In this and all subsequent experiments the sample size was reduced to 50 ml., the volumes of reagents being reduced to correspond. The smaller sample is sufficient for the 4-cm. Spekker cell which, it was now apparent, would be used in the determination, and tubes suitable for collecting this small volume of water were constructed.

TABLE I

EFFECT OF DIFFERENT METHODS OF ADDING STARCH SOLUTION

Method of addition of starch	Drum reading	Equivalent dissolved oxygen ml. per litre
Starch sample 1.		
A. Blown in rapidly from a pipette, then mixed by swirling	0·92, 0·90	0·009, 0·011
B. Run in from pipette with jet immersed, then mixed by swirling	0·91, 0·89	0·010, 0·012
C. Mixed by swirling during dropwise addition	0·89	0·012
Starch sample 2.		
A. Blown in rapidly from a pipette, then mixed by swirling	0·80	0·020
C. Mixed by swirling during dropwise addition	0·80	0·020

Quality of starch—In order to determine whether the position and slope of the calibration curve depends upon the batch of starch being used, six groups of three blank solutions, containing added iodine corresponding to 0·08, 0·05 and 0·03 ml. of oxygen per litre, were treated with starch solutions prepared from six different samples of soluble starch. The absorptions were measured and the linear parts of the calibration curves drawn (Fig. 3). Two batches of soluble starch over two years old, supplied by one manufacturer (*a*), gave a common curve *A* and a third batch from the same manufacturer, about one year old, gave the nearly parallel curve *B*. Using the difference method described on p. 348, the maximum error involved in reading from a single calibration curve for all three of these samples would be 0·002 ml. of oxygen per litre. Greater error will be probable if another manufacturer's soluble starch is used in conjunction with an existing calibration—see curves *C* and *D*, Fig. 3. It is strongly advised, therefore, that one bottle of soluble starch should be reserved for use in the dissolved oxygen determination and that when a fresh bottle is opened a new curve should be drawn.

Time of reaction—Bates, French and Rundle[16] studied the starch-iodide reaction by means of potentiometric titrations and observed that the activity of an iodine solution added to starch solution continues to decrease for several minutes. Talbot *et alia*[20] found that the colour was stable for at least 10 minutes but that it may fade after that time; on the other hand, Turner[12] claimed that there was no change during one hour. To a blank solution at 15° C. sufficient iodine, together with the appropriate amount of starch, was added to give a drum reading near the middle of the scale and the absorption was measured at known time intervals. The results obtained in duplicate experiments are shown in Fig. 4, from which it is seen that a constant reading is obtained between 2 and 5 minutes after mixing; for routine analyses a period of colour development of 5 minutes was selected.

Temperature effects—It has long been known that the colour of the starch-iodide complex is destroyed on warming the solution; Turner[12] reports that the fading is only slight at 60° C. and the colour disappears at about 70° C.; Talbot *et alia*[20] state that fading is perceptible at about 17° C. The effect of temperature under the conditions obtaining in the present method of determination was examined by adding to a number of blank solutions equal amounts of iodine, to give, on the addition of starch at 15° C., a drum reading near the centre of the scale. The temperatures of these solutions were adjusted to cover the range 5° to 50° C. before the starch was added; the absorptions were measured at the appropriate temperatures 5 minutes after the starch additions. (Sendroy[21] has stated that both the temperatures of mixing and of colour measurement should be controlled.) The results are shown in Fig. 5: since the blank solution without added iodine gave a drum reading 1·00 (at 15° C.) it will be appreciated that at 50° C. the starch-iodide complex had become almost colourless. It is possible that the above-mentioned differences between the various observers is due to an effect noticed by Pickering,[26] *viz.*, the temperature at which the colour disappears is dependent upon its intensity at ordinary temperatures. This feature was not examined experimentally because in the present method the range of iodine concentrations is small and the single set of observations near its centre should suffice. For routine determinations a temperature of 15° C. was selected as being both convenient and satisfactory.

Quantity of reagents—That the results obtained by the present method are not affected appreciably by possible inaccuracies in the amounts of alkaline iodide reagent, manganous sulphate or sulphuric acid added was proved by halving and doubling the normal quantities. This test was made with blank solutions containing added iodine at four concentrations and the results are given in Table II.

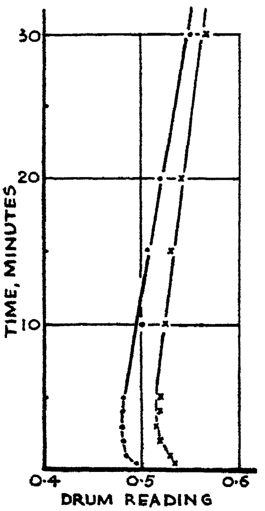

Fig. 4. Effect of time of colour development.

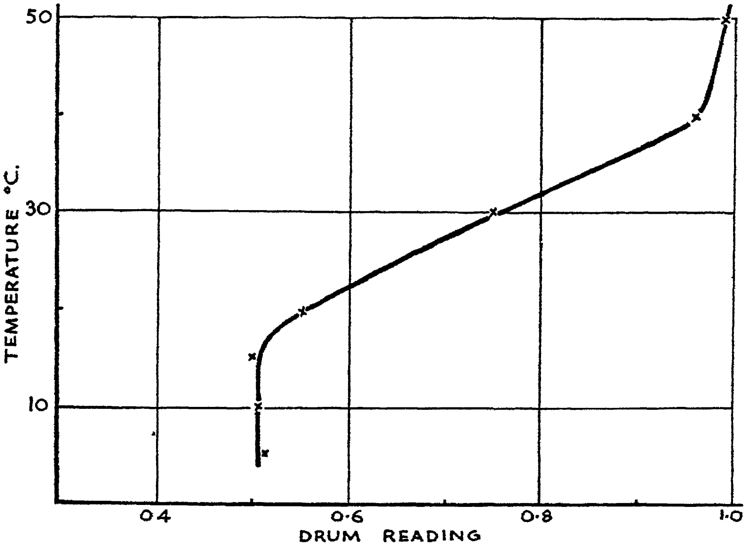

Fig. 5. Effect of temperature.

TABLE II

EFFECT OF REAGENT CONCENTRATIONS

Iodine added equiv. to dissolved oxygen, ml. per litre	Drum readings for reagent concentration: × 0·5	Normal	× 2
0·01	0·93	0·93	0·91
0·03	0·70	0·71	0·69
0·06	0·35	0·36	0·35
0·08	0·12	0·13	0·12

The effect of variations likely to occur during routine determinations is obviously negligible. The reproducibility of the method was examined by taking several groups of three samples from one source at the same time and measuring the absorptions after development of the starch-iodide complex as described on p. 348. Typical results are given in Table III.

TABLE III

REPRODUCIBILITY OF RESULTS

Sample No.	Drum reading	Equivalent dissolved oxygen, ml. per litre
11 A, B, C	0·75, 0·73, 0·74	0·024, 0·026, 0·025
12 A, B, C	0·84, 0·82, 0·82	0·016, 0·018, 0·018
13 A, B, C	0·79, 0·77, 0·78	0·021, 0·023, 0·022

Calibration—A calibration curve for routine use was constructed by measuring the absorptions of a series of blank solutions (50 ml. each) to which were added 1 ml. portions of iodine solutions of concentrations to correspond to approximately 0·01, 0·02, 0·03, 0·06, 0·08 and 0·09 ml. of oxygen per litre when calculated on 50 ml., and 1 ml. of 1 per cent. starch solution. In this way solutions were obtained each having a total volume of 52 ml., *i.e.*, the same as in the routine method (see p. 348). The various iodine solutions were prepared by diluting 0·1 *N* iodine and their strengths were determined accurately by titration with 0·01 *N* sodium thiosulphate. The colours were developed for 5 minutes at 15° C. and the absorptions measured at the same temperature. The curve AA of Fig. 6 is drawn through four series of values obtained in this manner; it is linear over most of the range, but becomes curved when the oxygen concentration exceeds about 0·08 ml. per litre. For each of the above series of iodine concentrations six sample tubes were connected in series and filled with feed water, thus ensuring that each contained the same concentration of interfering substances. If a considerable period of time elapses between the collection of these groups of samples, changes in the interfering substances may occur and different amounts of iodine may be absorbed or liberated. For example, in one of the sets of determinations plotted on curve AA the absorptiometer drum readings corresponded originally to dissolved oxygen contents uniformly 0·003 ml. per litre greater than those shown. The adjustment made to this series is justified because the procedure for routine determinations, described on p. 348, measures the effect due to interfering substances and corrects the apparent oxygen content of the water accordingly; it is, therefore, a difference method and the slope of the calibration curve, and not its true position, is important. It is obvious that because of this fact only the linear portion of the curve should be used; it is also apparent that the determination of the effect of interfering substances is essential if accurate results are to be obtained. The linear part of the calibration curve may be extended by using a mercury vapour lamp instead of the tungsten filament type: the straight line BB of Fig. 6 was obtained in this manner, using heat filters H503 and spectrum yellow filters H606 in conjunction with a 4-cm. cell.

The following experiment shows that the blank solution used for constructing a calibration curve must be freshly prepared. A blank solution was prepared in the usual manner by collecting feed water under oxygen-free conditions and adding the Winkler reagents in the order: alkaline iodide, sulphuric acid and manganous sulphate. To 50 ml. aliquots of this solution were added 1-ml. portions of starch solution after intervals of 0, 1 and 2 hours; the absorptiometer drum readings obtained were 0·96, 0·94 and 0·90 respectively, showing that there is a slow, but progressive, liberation of iodine.

Interfering substances—When determining dissolved oxygen it is not practicable to eliminate the effects of interfering substances as was done by Turner,[12] who used water freshly distilled from caustic potash. Also the procedure of Sendroy[21] is inapplicable: he found that there were slight changes in the position of the calibration curve when different supplies of distilled water were used, but he did not ascertain the cause. Each time an analysis was made Sendroy fixed the position of the calibration curve by the determination of two points on it. Both of these authors measured the depth of the blue colour formed by iodine that had been isolated by distillation. This difficulty is avoided by taking two samples of the feed water simultaneously and treating them as follows. To the first sample (called the *oxygen sample*) add the Winkler reagents in the usual manner, followed by a known volume of iodine solution.* Under these conditions both the dissolved oxygen and the interfering substances react. To the second sample (called the *interference sample*) add the reagents in the order alkaline iodide, sulphuric acid and manganous sulphate, and then add the same volume of iodine solution as was added to the oxygen sample. Under these conditions manganous hydroxide is not formed and so dissolved oxygen cannot react, but the interfering substances take effect. The true dissolved oxygen content of the water may, therefore, be found from the difference between the iodine concentrations determined in the two samples. This statement should be qualified in that no allowance is made for the oxygen which is dissolved in the reagents and which is active only in the oxygen sample; it can be calculated that with the volumes of reagents and sample used in the present method this interference corresponds to approximately 0·005 ml. per litre, a figure which should be deducted from the experimental results. Sillars and Silver[27] determine the extent of this interference by the use of an extra sample in every analysis. One sample is treated as usual and to the other the Winkler reagents are added in double the normal amounts; the oxygen derived from the reagents is then found by the difference between the results. Sebald[4] uses equal amounts of the same reagents in both samples, but one of the latter has double the volume of the other.

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* Schwartz and Gurney[29] appear to have been the first to add iodine to enable a correction to be made for the presence of interfering substances; these authors used potassium bi-iodate, which reacts with the iodide present in the sample. However, they made the addition only when no blue colour was developed in the normal procedure.

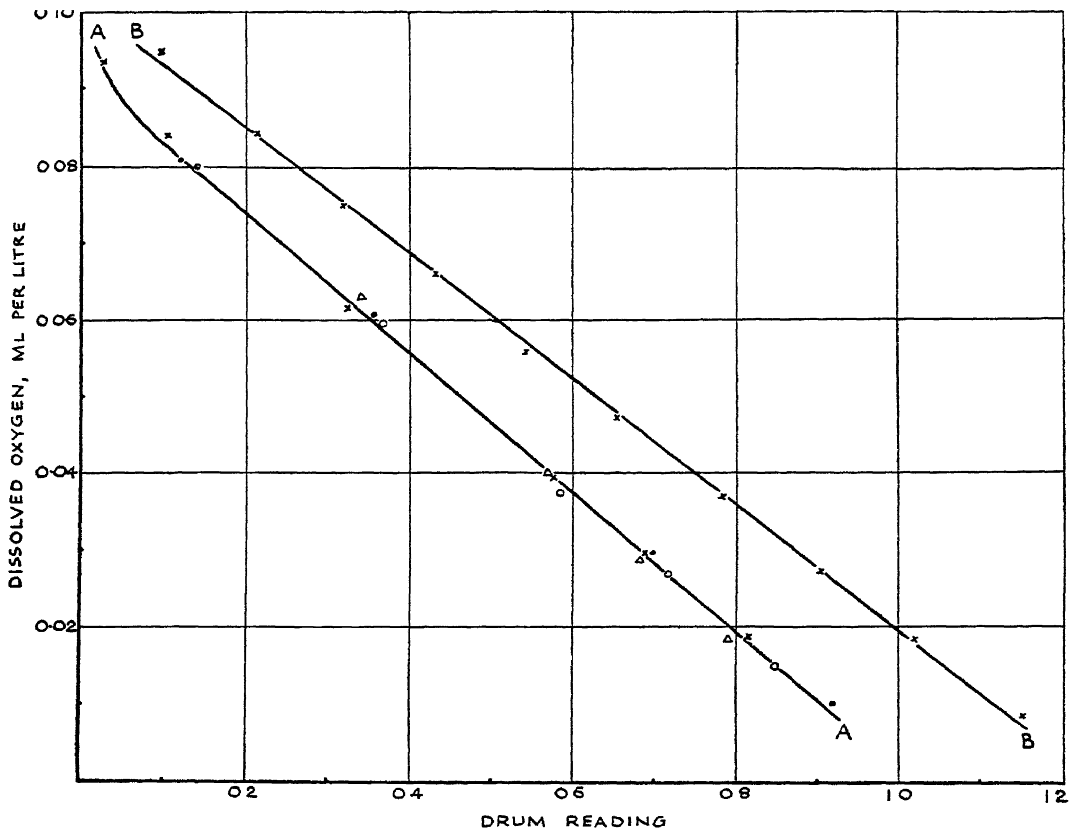

Fig 6 Calibration Curves.

AA — Tungsten filament lamp BB — Mercury vapour lamp.

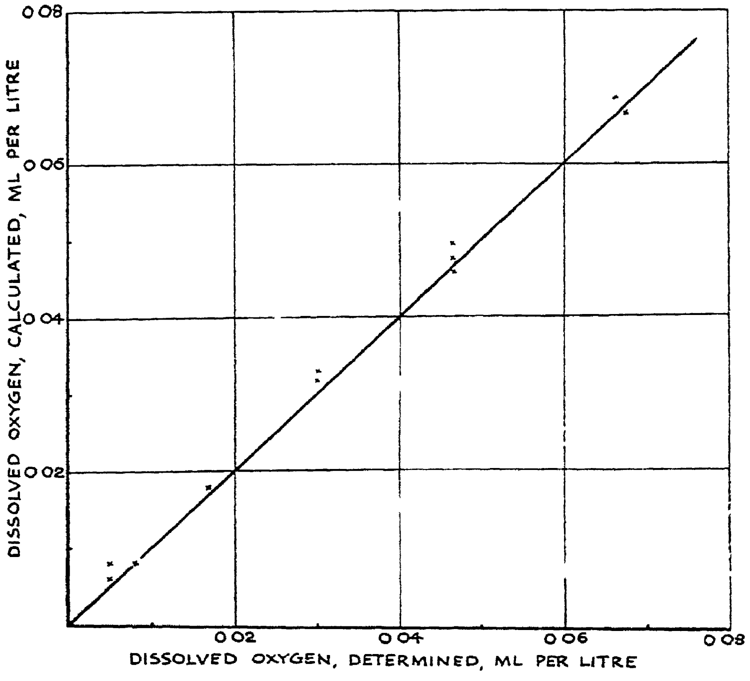

Fig. 7.

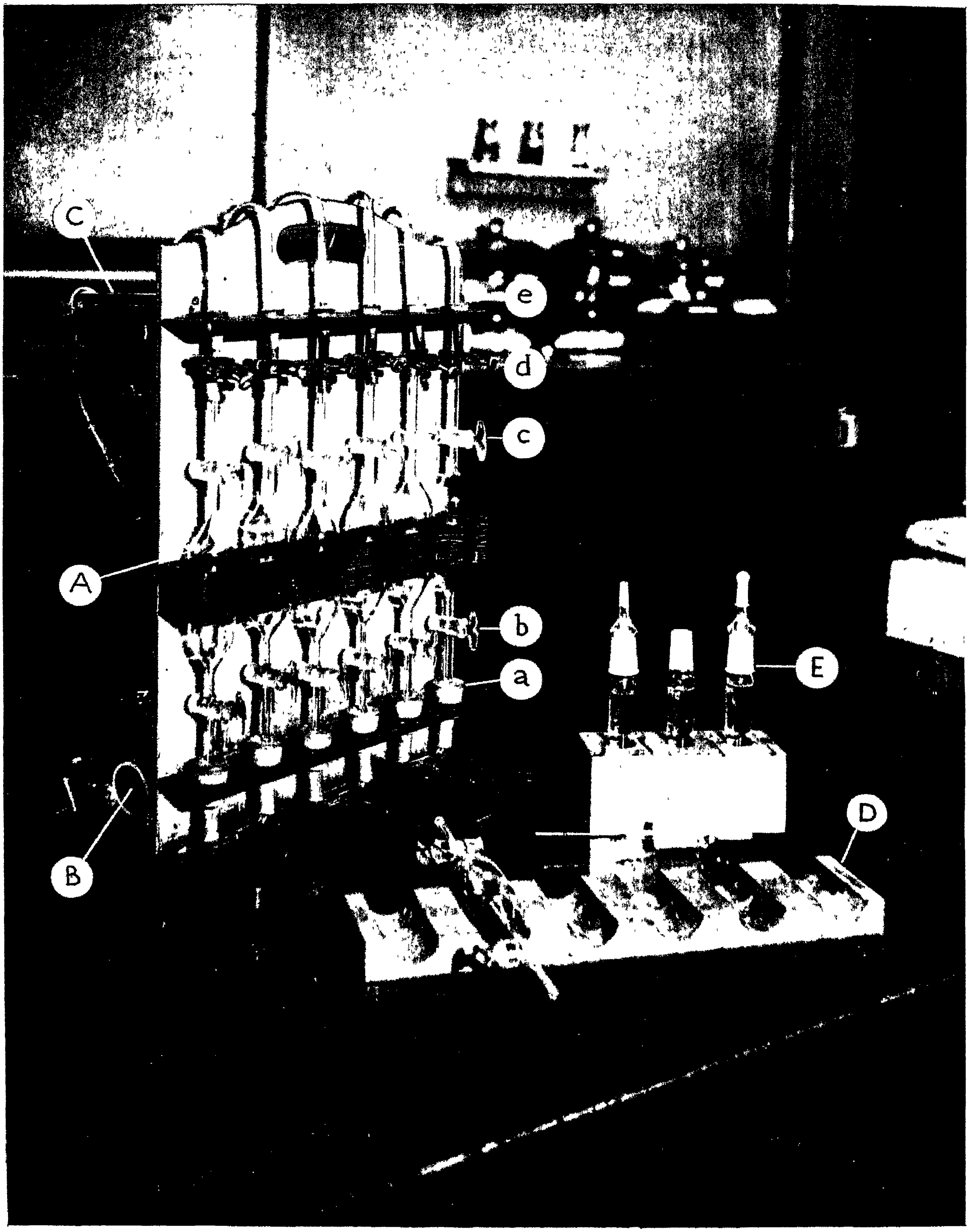

Fig. 8. Sampling Apparatus.

Finally, the method was tested by addition of known quantities of fully aerated water to samples of feed water and comparison of the calculated increases in dissolved oxygen content with the experimental values. The oxygen content of the aerated water was determined by the Winkler titration method and the desired volume of it was added to one of a pair of samples of feed water that had been collected simultaneously. The results are shown graphically in Fig. 7, which also shows the theoretical line. The maximum difference between the pairs of values is 0·003 ml. per litre, which is regarded as satisfactory since it includes all errors of sampling, manipulation and calibration.

The method which follows covers the range 0 to approximately 0·08 ml. of oxygen per litre when allowance is made for the iodine added to the interference sample. This is sufficient for the majority of determinations required in the control of feed waters and condensates for high pressure boilers, but occasionally it is necessary to determine higher concentrations. Experiments have shown that the sample may be treated with the Winkler reagents and diluted to twice the original volume either immediately before or after the addition of the starch solution and that the approximate results so obtained are of sufficient accuracy for usual requirements. The calibration curve for a mercury vapour lamp is, however, linear (BB, Fig. 6) and may be used in conjunction with 1-cm. or 0·5-cm. cells to cover the ranges 0 to 0·4 and 0 to 0·8 ml. of oxygen per litre respectively, but it is very improbable that the higher range would be necessary.

Method

Sampling apparatus—

The apparatus officially recommended[28] for the Winkler method has a capacity of 500 ml. The present authors previously used 250-ml. samples for this titration. The 4-cm. Spekker cell, however, has a capacity of about 30 ml. and it is therefore unnecessary to collect such large samples for the present method. This is an important consideration when an interference sample has also to be taken from each source and all waters for routine testing have to be carried to the laboratory. The equipment shown in Fig. 8 was therefore designed; it can accommodate six tubes *A* of capacity 55 ml. for three dissolved oxygen and interference samples, three Spekker flow-through cells *B* for *p*H measurements, and three 100-ml. bottles for conductivity samples (these are obscured in the photograph). The sample tubes for the oxygen determination resemble those described by Sebald[4] but are much smaller. Two graduations are etched on each of the narrow stems beyond the taps, the capacity between these marks being 0·15 ml., *i.e.*, the volume of reagents to be added to the contents. The lower graduation is situated a few mm. above the tap to leave a safety margin so that air is not admitted with the reagents. The lower ends of each pair of sample tubes are joined within rubber bungs *a*, held in the wooden frame, to a T-piece through which they are connected with the source to be sampled: a cooling coil should be used. The upper ends of the sample tubes are connected through rubber tubing carrying screw-clips *d* to outlet tubes, which are secured by rubber bungs *e* fixed in the wooden frame and which discharge into a metal tank *C*. The dimensions of the tank are 12 × 3 × 3 in., and it will accommodate water equivalent to the ten volume changes which it is necessary to run through the sample tubes in order to ensure thorough washing.

Collecting the sample—With clips *d* and taps *b* and *c* open, run the water through the sample tubes until tank *C* is almost filled. Reduce the rate of flow, close tap *b* and then screw-clip *d* on the tube that is to be used for the oxygen sample; all the water now passes through the tube to be used for the interference sample, on which tap *b* and screw-clip *d* are closed immediately after cutting off the water supply. Taps *c* are left open until the determination is made; contraction in volume due to fall in temperature will not then cause ingress of air because volume changes are accommodated by the flexibility of the rubber tubing. If cooling of the water below the prevailing temperature can be arranged, both taps *b* and *c* may be closed and the extra complication of the rubber tubing and screw-clips *d* may be avoided. The rubber tubing has not caused contamination of the samples and consequent erroneous results.

Reagents—

Alkaline iodide solution—Measure 250 ml. of distilled water: in part dissolve 35 g. of potassium iodide (AnalaR); in the remainder dissolve 150 g. of sodium hydroxide (AnalaR, stick). Mix the two solutions.

Manganous sulphate solution—Dissolve 113 g. of $MnSO_4.4H_2O$ (AnalaR) in 250 ml. of distilled water.

Diluted sulphuric acid (1 + 1).

Iodine solution—Approximately 0·0002 *N*: prepared by dilution of 0·1 *N* solution.

Starch solution—To 90 ml. of boiling distilled water add from a test tube a suspension of 1 g. of soluble starch (AnalaR) in 5 ml. of water; rinse the test tube with 5 ml. of water. Boil the solution for precisely one minute, cool and make up to 100 ml. with water. This solution must not be used when more than a week old.

PROCEDURE—

Close taps *c* and remove the rubber tubing and screw-clips *d* from the sample tubes; withdraw the latter from the bungs *a* and place on the wooden block *D*, which forms a safe and convenient stand for the tubes between the steps of the following procedure. The sample tubes do not carry distinguishing marks, but no confusion arises if they are kept in the same order on the sampling stand and on block *D*, *viz.*:

Sample 1		Sample 2		Sample 3	
Oxygen tube	Interference tube	Oxygen tube	Interference tube	Oxygen tube	Interference tube

Treat each sample tube in rotation as follows. Remove water remaining in the stem *d* by means of a glass jet connected to a water pump and fill stem *d* with alkaline iodide reagent from the appropriate dropper *E*, the container for which is kept filled with solution so that the dropper fills without application of suction. Withdraw excess reagent by means of the glass jet until the meniscus is at the outer of the two graduations. Hold the tube vertically with end *d* uppermost, open tap *c* and then cautiously open tap *b* until the reagent meniscus falls to the lower graduation; close taps *b* and *c* in that order (0·15 ml. of reagent has then been added to the sample). By means of a second jet rinse out reagent remaining in stem *d*. Mix the contents of the sample tube by rocking movements and replace it on block *D* in the reverse position from that which it formerly occupied. Add subsequent reagents in a similar manner, but use stem *a* to avoid mixing with alkaline iodide solution remaining in the bore of tap *c*. The oxygen samples are treated with manganous sulphate solution* followed by sulphuric acid: the same reagents are added in the inverse order to the interference samples. Both oxygen and interference samples may be exposed to atmospheric oxygen without affecting the results after complete solution of the manganese hydroxide precipitate has taken place; both are treated as follows. Hold the sample tube vertically with stem *a* uppermost; open the upper tap and rinse out the lower stem with a few ml. of the contents by opening the lower tap. Measure 50 ml. of the solution in a graduated flask and transfer to a conical flask, cool to 15° C. and add 1 ml. of 0·0002 *N* iodine and 1 ml. of starch solution. After leaving for 5 minutes for the blue colour to develop, measure the absorptions, using 4-cm. cells with heat filters H503 and spectrum yellow filters H606 in conjunction with a mercury vapour lamp; drum setting, water - water, 1·20. From the calibration curve read the oxygen equivalents of the absorptions of the oxygen and interference samples and from the difference between these values deduct 0·005 ml. per litre to correct for the oxygen dissolved in the reagents; the result is the true dissolved oxygen content of the water. When the samples have been collected, the determination of the dissolved oxygen in three sources of water occupies about 30 minutes.

SUMMARY

Existing methods for the determination of oxygen dissolved in feed waters and condensates are critically reviewed and a more convenient, yet accurate, procedure is proposed. The new method depends upon the measurement of the intensity of the blue starch-iodide colour by means of the Spekker photo-electric absorptiometer. A 55-ml. sample suffices for the determination and apparatus has been devised for its collection.

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* The oxidation of manganous hydroxide is comparatively slow, but the reaction is completed if the addition of the sulphuric acid is made about 3 minutes after that of the manganous sulphate.

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RESEARCH DEPARTMENT
STONEBRIDGE PARK

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The Determination of Nitrates in Water

BY F. W. ALLERTON

THE various methods available for the determination of nitrates in water fall into three groups: (*a*) nitrometer methods; (*b*) direct colorimetric estimations; (*c*) reduction methods with subsequent colorimetric estimation.

Of the first group, Crum's nitrometer method is the only one of importance and it has long been superseded by safer and more rapid methods. The second group includes the phenol-sulphonic acid and indigo-carmine methods. The former, depending upon the production of picric acid and subsequent colorimetric estimation, is by no means rapid and is influenced by any considerable amount of chlorides present in the water; furthermore, the evaporation of the sample to dryness may involve losses of nitrogen. The indigo-carmine method based upon the oxidation of the dye to a colourless compound is largely used, but is stated by Sutton[1] to give approximate results only. Both of these colorimetric methods are criticised by Thresh[2], who states that reduction methods yield the most satisfactory results, especially when the reduced nitrogen is separated by distillation prior to nesslerisation.

Thresh reduces the nitrates in faintly acid solution for one hour with zinc-copper couple and subsequently distils. Free ammonia is first removed from the sodium hydroxide or carbonate employed by boiling, and the zinc-copper couple is carefully excluded from the distillation flask, presumably to prevent reduction of any nitrates contained in the alkali.

I have found this method to give satisfactory results, but the preparation of the zinc-copper couple and the reduction period needed detract from the method as a routine laboratory process.

In an attempt to overcome these disadvantages the possibility of using Devarda alloy was investigated. This alloy has been used for a number of years for the routine estimation of nitrates in manures, and Donald[3] has made a thorough investigation of the best conditions for dealing with quantities of sodium nitrate of the order of 1 g. There appears, however, to have been no adaptation of this method to quantities of nitrogen of the order of 0·0001 g., such as are found in convenient quantities of most natural waters.

Apparatus—For this purpose, the following simple apparatus was used. A 250-ml. flask with ground-glass delivery was connected to a vertical water condenser, the whole being assembled so that a 100-ml. Nessler glass could be adjusted as required beneath the delivery of the condenser.

Experimental—As a preliminary trial, half the quantities of ammonia-free water, A.R. sodium hydroxide and Devarda alloy recommended by Donald were used and nitrate equivalent to 0·00005 g. of nitrogen was taken for estimation. The standard nitrate solution was prepared from A.R. potassium nitrate and contained 0·00001 g. of nitric nitrogen per ml. Despite careful heating, frothing was so marked that a satisfactory distillation was impossible. Consequently, the effect of smaller amounts of alloy and alkali was investigated.

With 0·5 g. of alloy and an amount of sodium hydroxide necessary to give a concentration of 0·05 N there was still considerable frothing, but careful heating enabled a normal distillation to be carried out. By nesslerising an aliquot portion it was found that 0·00007 g. of nitrogen was present in the distillate. There was, therefore, despite the use of A.R. sodium hydroxide, a very large error due to ammonia or nitrates in the reagents.

In order to locate the source of the ammonia, a 5 per cent. sodium hydroxide solution was prepared from carefully cleaned sodium metal and ammonia-free water. When this was used the distillate contained 0·000062 g. of nitrogen, which, since the caustic soda could be assumed nitrogen-free, pointed to the presence of nitrogen in the Devarda alloy. A blank test confirmed this conclusion.

A quantity of the alloy was therefore boiled with three successive portions of 100 ml. of distilled water and then carefully washed on a filter with ammonia-free water. An estimation with 0·00005 g. nitrogen and the washed and dried alloy (0·5 g.) gave 0·0000529 g. of nitrogen.

It seemed probable that the error could be still further decreased and the frothing diminished by using smaller quantities of alloy and alkali.

At this point, the following technique was adopted—

The required number of ml. of standard nitrate solution were pipetted into the 250-ml. flask, and a known volume of the 5 per cent. sodium hydroxide solution was added. Amounts of Devarda alloy ranging from 0·20 g. to 0·5 g. were then added and the volume was made up to 120 ml. with ammonia-free water. The distillation was regulated so that about 15 minutes elapsed before the flask boiled, in order to prolong the reduction period. Fifty ml. of distillate were now rapidly collected. Since at the beginning of heating an appreciable volume of hydrogen was evolved, which, it was thought, might carry over ammonia gas, the end of the condenser was made to dip into 2 ml. of ammonia-free water in the Nessler cylinder. After a few minutes the cylinder was lowered into the normal position.

Tables I and II illustrate the results of using various amounts of nitrogen as nitrates with different concentrations of alkali and weights of Devarda alloy.

TABLE I

Devarda alloy, 0·25 g.; water, 120 ml.

Nitric nitrogen taken g.	Nitrogen found, with 0·1 N NaOH g.	Nitrogen found, with 0·05 N NaOH g.	Volume distilled ml.
0·00005	0·0000501	0·0000491	50
	0·0000502	0·0000494	
0·00010	0·0000992	0·0000982	50
	0·0000996	0·0000980	
0·00020	0·0002036	—	50
	0·0002020		
0·00050	0·000498	0·000489	100
0·00100	0·000963	0·000976	100

It will be seen from Tables I and II that—

(1) 0·05 N sodium hydroxide is inclined to give incomplete reduction, especially of the larger amounts of nitrates; and

(2) there is no advantage in using 0·5 g. of Devarda alloy for less than 0·00020 g. of nitrogen.

With smaller quantities of nitrogen, where a larger proportion of the distillate is nesslerised, 0·5 g. of alloy appears to give slightly high results owing to remaining traces of ammonia or nitrates as impurity.

TABLE II

Devarda alloy, 0·50 g.; water, 120 ml.

Nitric nitrogen taken g.	Nitrogen found, with 0·1 *N* NaOH g.	Nitrogen found, with 0·05 *N* NaOH g.	Volume distilled ml.
0·00005	0·0000502	0·0000496	50
0·00010	0·000101	0·0000988	50
0·00020	0·000206	0·000196	50
0·00050	0·000500	0·000494	100
0·00100	0·00105	0·000975	100

With quantities of nitrogen from 0·00020 g. to 0·0010 g. the ammonia was not completely distilled into 50 ml. and a further 50 ml. was necessary. It is apparent that a balance must be struck between excess ammonia due to impurities in the reagents and incomplete reduction of the nitrates to ammonia.

With quantities of nitrogen above 0·00020 g. a small proportion only of the distillate is nesslerised and hence the influence of reagent impurities is negligible with reagents prepared as stated. Rather higher concentrations of alkali might be used to ensure complete reduction in a shorter time, but I have found that the advantages so gained are more than offset by increased frothing.

In order to test the method without the bias possible from the use of known quantities of nitrogen, an assistant was instructed to add an amount of the standard solution between 5 ml. and 20 ml. to a flask containing 0·25 g. of Devarda alloy and 120 ml. of 0·1 *N* sodium hydroxide. The amount found was 9·3 ml. instead of 9·2 ml. added—an error of little more than 1 per cent.

SUMMARY

The method described is claimed to be rapid and accurate providing that the reagents are especially freed from nitrates and ammonia. The most suitable quantities appear to be: nitrate nitrogen 0·000050 to 0·00020 g., Devarda's alloy 0·25 g. and sodium hydroxide concentration 0·1 *N*.

Thus, in 10 ml. of waters containing from 0·5 to 2·0 parts of nitrate nitrogen per 100,000, the amount can be estimated at least within the accepted accuracy of the Nessler method.

Note—It is of course assumed that an allowance is made for free ammonia and nitrites in waters abnormally high in these substances.

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51 BROWNING AVENUE
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The Analysis of Nickel - Cobalt - Iron Alloys Used in Glass-to-Metal Seals*

BY R. C. CHIRNSIDE, H. J. CLULEY, AND P. M. C. PROFFITT

INTRODUCTION—

THE methods for the construction of many modern thermionic devices involve the use of a glass-to-metal seal. Nickel - iron alloys have been used for this purpose for many years where the use of "soft" glasses was permissible. In many of the modern applications it is necessary to use low-expansion glasses and, for sealing to these glasses, the metals molybdenum and tungsten have been used. Apart from the cost, which is high, there are other features of the use of these two metals which do not make them wholly desirable and new low-expansion alloys have been developed for the purpose. These new alloys contain nickel, iron and cobalt as their main constituents and have become well known in the U.S.A. under the commercial names "Kovar" and "Fernico."

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* Communication from the Staff of the Research Laboratories of The General Electric Company Limited, England.

The need for these materials greatly increased during the war and their manufacture and their use in this country have already reached considerable proportions. It is essential for the manufacture of good seals that the physical properties of the alloy, more especially the expansion characteristics, shall be closely controlled and, although the only completely satisfactory check is by making a test seal, the alloy manufacturer and the user have both been concerned to set up some specification of chemical composition. Specifications were drawn up in the U.S.A. and in this country, but there was some suggestion, certainly in America and possibly here also, that the tolerances allowed were not as great as the available analytical methods required. Indeed, in these laboratories for some time it was considered inadvisable to make use of the analytical figures obtained, as the extent of their error was unknown.

This paper discusses the analytical problem, makes some reference to the experimental difficulties and goes on to describe the methods that have been found satisfactory for the accurate analysis of this type of alloy.

THE ANALYTICAL PROBLEM—

The specifications for the American and the British alloy compositions are given in Table I, and it will be seen that in the British specification the "nickel plus cobalt" content is specified with a tolerance of ± 0·5 per cent. and the nickel alone with the same tolerance. In the American specification the nickel, iron and cobalt are all given as ranges, the extremes of which add up to from 98·9 to 100·4.

The form in which these figures are presented suggests that the analytical methods available do not give results of high precision.

TABLE I

	American	British
Nickel	28·7–29·2 per cent.	29 ± 0·5 per cent.
Cobalt	17·3–17·8 "	17 "
Nickel + cobalt	—	46 ± 0·5 "
Iron	52·9–53·4 "	—
Manganese	0·50 max. "	0·3 "
Silicon	0·20 max. "	0·15 "
Carbon	0·06 max. "	0·05 max. "
Sulphur	—	0·01 max. "
Phosphorus	—	0·01 max. "

With iron, nickel and cobalt all present in substantial proportions, the analytical problem may be stated thus:—

(1) Nickel and cobalt are not quantitatively separated by dimethylglyoxime or α-nitroso-β-naphthol, without the use of special methods.

(2) A clean separation of iron from nickel and cobalt is not achieved by the usual precipitants such as ammonia or zinc oxide, and a new problem is introduced in that the presence of iron further complicates the separation of nickel from cobalt.

Attention was therefore initially directed to recently published work on analogous problems.

EXPERIMENTAL

(i) Using synthetic solutions containing iron, nickel and cobalt to simulate the composition of the alloys, the scheme outlined by B. S. Evans[1] was investigated. This depends on the precipitation of nickel and cobalt as cobalticyanides to separate these metals from iron, and the subsequent determination by cyanide titration of nickel, and of nickel plus cobalt. The cobalt is thus obtained as the difference between two determinations.

Various difficulties were encountered, but the principal objection to the method, and the one that led to its ultimate rejection, was the nature of the cobalticyanide precipitate, which was always difficult and often impossible to filter. It was clear that the method was not directly applicable to alloys of the particular composition with which we were concerned.

(ii) The next experiments were concerned with adapting the methods of W. R. Schoeller[2] to our particular problem. Schoeller's scheme involves the separation of iron as ferric phosphate in acetic acid solution; subsequent precipitation of the cobalt, together with some of the nickel, as cobalt ammonium phosphate; and determination by cyanide titration of the nickel in the main portion and also of the nickel co-precipitated with the cobalt. Here again the cobalt is obtained as the difference between two determinations.

Applying these methods to synthetic solutions whose composition simulated the alloys it was not found possible to obtain clean separations, even with reprecipitations, and as was also the case with Evans's method, it was impossible to achieve any sort of precision, let alone accuracy, for the determinations. Thus results obtained for nickel in a number of experiments varied from 29 to 31 per cent. and for cobalt from 14 to 19 per cent.

(iii) DETERMINATION OF NICKEL—

In view of these difficulties, attention was redirected to the determination of nickel with dimethylglyoxime in presence of iron and cobalt.

Although bivalent cobalt forms complexes with dimethylglyoxime, these are soluble and nickel may be satisfactorily determined in presence of cobalt alone provided that sufficient dimethylglyoxime is added to combine with both metals. An alternative method is to oxidise the cobalt with hydrogen peroxide in ammoniacal solution, destroy the excess hydrogen peroxide by boiling and precipitate the nickel with dimethylglyoxime, with which the oxidised cobalt does not combine. Nickel can also be determined in presence of iron, using tartrate or citrate to prevent precipitation of iron in ammoniacal solution. The ease with which separation of either of these binary mixtures may be made will depend, for any particular mixture, on the relative proportions of the constituents. When nickel is present with both cobalt and iron a new problem arises and the relative proportions of the three metals as they occur in the Kovar type of alloy appear to raise the problem in a very acute form. Thus, when an attempt is made to precipitate nickel with dimethylglyoxime from such a mixture in the usual manner, brown slimy precipitates difficult to filter are produced, the nickel compound being grossly contaminated with both cobalt and iron. The difficulty cannot be overcome by oxidation of the cobalt as mentioned above, because this method is not effective in presence of tartrate.

Examination of the contaminated precipitates by X-ray diffraction methods showed that a distinct second phase was present in addition to the nickel dimethylglyoxime complex. A search of the literature indicated that the contaminant might be the compound reported by J. G. Weeldenburg,[3] and said to contain one atom of iron, one of cobalt and three molecules of dimethylglyoxime. The formation of this compound will be discussed later in the paper.

Methods for preventing the co-precipitation of iron and cobalt were suggested as long ago as 1913 by O. Brunck.[4,5] These methods were rediscovered 24 years later by G. Balz,[6] as pointed out by H. Diehl.[7] It was claimed that the iron - cobalt - dimethylglyoxime compound was formed only by ferric iron, and that the formation of this compound could be prevented by reduction of iron to the ferrous state. The next experiments were therefore directed along these lines.

We were able to confirm Balz's findings that the separation is improved by (1) preliminary reduction of the iron with sodium sulphite, (2) neutralisation with sodium acetate instead of ammonia after addition of dimethylglyoxime to the acid solution, (3) precipitation at 70° C. instead of at the boiling point and (4) shorter times of standing.

The following method, based on these observations, affords a clean separation in a single precipitation. Examination by X-ray diffraction technique of precipitates obtained by this method indicated that the nickel dimethylglyoxime complex was the only phase present; chemical tests by colorimetric methods confirmed the absence of cobalt and iron.

METHOD FOR THE DETERMINATION OF NICKEL

Weigh 0·2 g. of the sample into a 400-ml. beaker, add 10 ml. of diluted nitric acid (1 + 1) and warm until it is dissolved.

Add 20 ml. of diluted sulphuric acid (1 + 1), evaporate to fuming to remove the nitric acid and fume for 5 minutes. Cool, add 75 ml. of water and heat to boiling to dissolve the salts. Filter, if necessary, through a No. 40 Whatman filter paper and wash with hot water. Cool somewhat, add diluted ammonia solution (2 + 1) until a slight permanent precipitate is formed and then clear with diluted sulphuric acid (1 + 6) followed by 20 drops in excess. Add 2·0 g. of sodium sulphite and boil for a few minutes to reduce $Fe^{\cdots}$ to $Fe^{\cdot\cdot}$. Cool, add 2·0 g. of tartaric acid, neutralise with ammonia and re-acidify with diluted sulphuric acid (1 + 6) adding 20 drops in excess.

Dilute to 250 ml., adjust the temperature to 70° C. and precipitate the nickel with 75 ml. of 1 per cent. alcoholic dimethylglyoxime, followed by 4 g. of sodium acetate crystals dissolved in a small volume of water, to neutralise the mineral acid and ensure complete precipitation of the nickel complex. Stir vigorously, and then allow to stand for 15 minutes on the bench.

Filter the precipitate through a weighed No. 3 grade sintered glass crucible and wash thoroughly with warm water. Suck as dry as possible, dry at 110° C. for 1 hour and re-weigh.

Table II shows results for nickel obtained on synthetic solutions made to correspond in composition to the alloys.

TABLE II

DETERMINATION OF NICKEL IN PRESENCE OF COBALT AND IRON

Iron present mg.	Cobalt present mg.	Nickel taken mg.	Nickel found mg.
106	34	59·88	59·80
”	”	59·88	60·04
”	”	59·88	59·94
”	”	59·88	59·72

(iv) DETERMINATION OF COBALT—

For the determination of cobalt in presence of iron and nickel the method of Dickens and Maassen[8] appeared to be promising. This consists in an electrometric titration of the cobalt in ammoniacal solution with potassium ferricyanide, which oxidises cobalt to the tervalent state; citrate is added to prevent precipitation of iron. The only common element that causes interference is manganese, which is present in these alloys as a minor constituent. In these circumstances the authors recommend addition of excess ferricyanide and back-titration with standard cobalt solution; by this method the sum of manganese plus cobalt is determined and the manganese is subsequently determined by another method. Tomicek and Frieberger[9] recommend the removal of manganese prior to titration of the cobalt, and Tomicek and Kalny[10] indicate that manganese can be oxidised to the tervalent or to the quadrivalent state, according to the conditions.

In absence of manganese the method of Dickens and Maassen worked admirably, but in presence of even small amounts of manganese the end-points were poor and considerable loss in accuracy resulted. It was therefore decided to remove manganese prior to titration and for this purpose the precipitation of manganese dioxide with nitric acid and potassium chlorate proved effective. With this method precipitation may fail for very small amounts of manganese (of the order of 1 mg.) but the method has always been found to work satisfactorily for quantities likely to cause significant errors in the cobalt determination. For the final titration of the cobalt a back-titration technique has been adopted, as better end-points are obtained in this way.

METHOD FOR THE DETERMINATION OF COBALT

Apparatus—For the electrometric titration the electrodes are (1) platinum electrode and (2) normal calomel electrode, which are connected to a galvanometer through the sliding contact of a potentiometer. The potentiometer is connected to a 1·5-volt cell in an external circuit and this arrangement permits rapid adjustment of the galvanometer by means of the potentiometer. A Tinsley portable galvanometer, full scale deflection ≡ 4 micro amps., was found to be of adequate sensitivity. A mechanical stirrer is used during the titration.

The circuit diagram is as shown in the figure (p. 355).

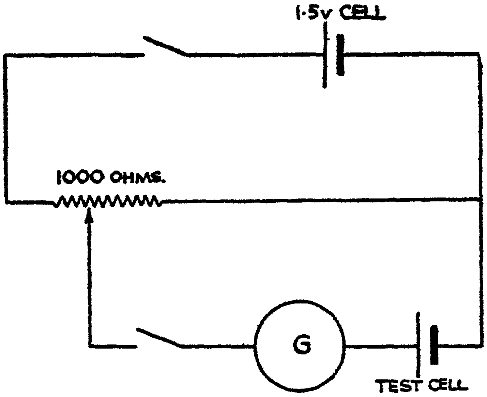
Reagents—(1) Cobalt nitrate solution: 10 g. of $Co(NO_3)_2.6H_2O$ per litre (1 ml. ≡ approx. 0·002 g. Co). Standardise the cobalt content by precipitating with α-nitroso-β-naphthol, ignite to oxide and reduce to metal in a stream of dry oxygen-free hydrogen.

(2) Potassium ferricyanide solution: 11·17 g. of $K_3Fe(CN)_6$ per litre. Standardise against the cobalt solution and adjust to equivalency. Store in a dark bottle and re-standardise periodically.

(3) Ammonium citrate solution: 300 g. per litre.

Standardisation of ferricyanide solution—Introduce 100 ml. of ammonium citrate solution and 50 ml. of "880" ammonia into a 600-ml. beaker and dilute to 300 ml. with water. The solution should be cool (about 15° C.). Add 25 ml. of the ferricyanide solution, immerse the electrodes and commence stirring. Adjust the galvanometer to zero and titrate the solution slowly with the cobalt nitrate solution. As the titration proceeds, the galvanometer spot light moves slowly across the scale and it will be necessary to re-adjust to zero to continue observation of the spot light. Continue the titration until one drop produces an observable deflection of the galvanometer. Continue the titration dropwise, observing visually the deflection produced for each drop added. The deflection per drop increases to a sharp maximum at the end-point, beyond which a sudden fall in the deflection per drop is observed. During the dropwise part of the titration it may again be necessary to adjust the galvanometer to zero.

Procedure—Dissolve 0·2 g. of the sample in 10 ml. of diluted nitric acid (1 + 1) in a 250-ml. beaker, and evaporate on the hot plate to a syrupy consistency. Add 100 ml. of nitric acid and boil to remove any oxides of nitrogen. Then remove the beaker from the source of heat and add, in small quantities, 5 g. of potassium chlorate with the aid of a glass spoon. During the addition the cover should be kept on as much as possible to minimise losses from the solution. When the addition is complete boil the solution vigorously until the volume is reduced to about 25 ml. Wash down the cover and sides of the beaker, add 40 ml. of water and cool quickly.



Filter the precipitate of manganese dioxide on filter paper pulp in a Gooch crucible, and wash with cold 2 per cent. nitric acid. Evaporate to remove the bulk of the nitric acid, add 100 ml. of water, warm to dissolve salts and cool.

Into a 600-ml. beaker introduce 100 ml. of ammonium citrate solution, 50 ml. of "880" ammonia, and 50 ml. of water, and cool the solution in running water. To this ammoniacal citrate solution add 25 ml. of the ferricyanide solution, and then slowly, and with stirring, add the sample solution. Immerse the electrodes, commence stirring, adjust the galvanometer to zero and titrate the excess of ferricyanide with cobalt nitrate solution, observing the end-point as for the standardisation.

If manganese is absent the separation with nitric acid and potassium chlorate may be omitted.

Results obtained on synthetic solutions by the method described above are shown in Table III.

TABLE III

DETERMINATION OF COBALT IN PRESENCE OF NICKEL AND IRON (MANGANESE PREVIOUSLY REMOVED AS MnO_2)

Iron present mg.	Nickel present mg.	Manganese present mg.	Cobalt taken mg.	Cobalt found mg.
62	34	0	20·0	20·0; 20·1
„	„	0·5	20·0	19·9; 20·2
„	„	1·0	20·0	20·0; 19·9
„	„	2·0	20·0	19·9; 20·0
„	„	5·0	20·0	20·2; 20·2

(v) DETERMINATION OF IRON—

It was considered desirable to determine all three major constituents of the alloys rather than employ the difference method, particularly in view of the presence of manganese and other minor constituents. The possibility of separating iron from nickel and cobalt to permit subsequent reduction and titration with dichromate was therefore investigated.

Precipitation with ammonia or zinc oxide, or with phosphate as in Schoeller's method, was not satisfactory even with reprecipitations. However, the classical "basic acetate" separation afforded a precipitate of iron almost completely free from nickel and cobalt, the amounts of these metals present being small enough to have no effect on the subsequent titration of the iron. A further reprecipitation with ammonia proved to be of little advantage, and a single "basic acetate" precipitation, carried out with care, gave satisfactory results.

METHOD FOR THE DETERMINATION OF IRON

Reagents—Ammonium carbonate solution: 20 per cent. w/v. Ammonium carbonate solution: 1 per cent. w/v. Acetic acid: 2 *N*. Ammonium acetate: 2 *N*. Stannous chloride solution: 5 g. dissolved in 7 ml. of hydrochloric acid and diluted to 100 ml. with water. Mercuric chloride solution: 5·6 per cent. w/v. Barium diphenylamine sulphonate solution: 0·15 per cent. w/v in water.

Procedure—Dissolve 0·5 g. of the sample in 20 ml. of diluted hydrochloric acid (1 + 1) and 5 ml. of diluted nitric acid (1 + 1) in a 600-ml. beaker. Evaporate nearly to dryness, add 10 ml. of concentrated hydrochloric acid and again evaporate nearly to dryness to remove the nitric acid. Add 10 ml. of diluted hydrochloric acid (1 + 4), warm to redissolve salts, dilute to 100 ml. and cool.

Add ammonium carbonate solution, drop by drop, using first the 20 per cent. solution and finally the 1 per cent. solution, until a faint precipitate not redissolving in 2 minutes is obtained. Add 3 ml. of 2 *N* acetic acid, dilute to 400 ml. with water and boil for 1 minute. (If the ammonium carbonate addition has been correctly carried out, the addition of acetic acid gives a clear solution, but, on heating, the bulk of the iron is precipitated before the boiling temperature is reached.) Add 8 ml. of 2 *N* ammonium acetate and again boil for 1 minute.

As soon as the precipitate has settled, filter through an 11-cm. No. 41 Whatman filter paper, washing at first with hot water containing 2 ml. of 2 *N* ammonium acetate and 1 ml. of 2 *N* acetic acid per 100 ml. and finally with hot water alone.

Return the precipitate and paper to the original beaker, add 30 ml. of diluted hydrochloric acid (1 + 1) and leave on the steam bath until the paper has disintegrated. Heat to boiling and add the stannous chloride solution dropwise until the yellow colour of the ferric chloride disappears and then add 5 drops in excess. Cool quickly, add 5 ml. of mercuric chloride solution and allow to stand for 1 minute. Dilute to 150 ml., add 10 ml. of phosphoric acid and 10 drops of barium diphenylamine sulphonate indicator and titrate with 0·1 *N* potassium dichromate until a purple colour is obtained.

Results obtained on synthetic solutions by the method described above are shown in Table IV.

TABLE IV

DETERMINATION OF IRON AFTER SEPARATION BY THE "BASIC ACETATE" PROCESS

Nickel present g.	Cobalt present g.	Iron taken g.	Iron found g.
0·138	0·081	0·2529	0·2545
"	"	0·2529	0·2534
"	"	0·2529	0·2529
"	"	0·2529	0·2542

(vi) DETERMINATION OF MANGANESE—

Preliminary experiments were directed towards a titration of the cobalt plus the manganese with ferricyanide followed by a separate determination of the manganese, the cobalt being determined by difference.

As mentioned under section (iv) on the determination of cobalt, it was found that a preliminary separation of the manganese gave more satisfactory results in the cobalt determination.

An absorptiometric method for the determination of manganese was therefore adopted. This made it possible to use a larger sample than would have been convenient if cobalt had also to be determined, and had the outstanding advantage of absorptiometric methods in that no preliminary separation of the manganese was necessary.

Method for the determination of manganese

Dissolve 0·5 g. of the alloy in 25 ml. of diluted nitric acid (1 + 1) in a 100-ml. beaker and heat to boiling to eliminate nitrous fumes. Add 5 ml. of phosphoric acid (to prevent subsequent precipitation of sparingly soluble ferric periodate), 10 ml. of water and 0·3 g. of potassium periodate. Heat to boiling and boil gently for 10 minutes to ensure complete oxidation of the manganese, cool, and make up to 100 ml. with water in a standard flask. Complete the determination with the Spekker absorptiometer as follows.

Fill a 1-cm. glass cell with the coloured solution, insert it in the instrument and place heat-absorbing and Ilford spectrum green filters in position. Set the drum to zero, switch on the tungsten lamp light source and adjust the left-hand iris until the galvanometer deflection is nil. To a second 1-cm. cell add 1 drop of 2 per cent. sodium nitrite solution followed by the test solution, allow time for the permanganate colour to be discharged and then insert in the "Spekker." Switch on the lamp and rotate the drum until a zero galvanometer deflection is obtained and take the drum reading.

Preparation of standards—

Pipette 10 ml. aliquots of each of the following three solutions into 100-ml. beakers to simulate Kovar type alloys. *Iron*—6·625 g. of pure iron dissolved in a minimum amount of diluted nitric acid (1 + 1) and diluted to 250 ml. *Cobalt*—10·6 g. of cobalt nitrate (AnalaR) dissolved in water and made up to 250 ml. *Nickel*—18·6 g. of nickel nitrate (AnalaR) dissolved in water and made up to 250 ml.

Prepare standard manganese solution by dissolving 0·25 g. of pure manganese metal in 5 ml. of diluted nitric acid (1 + 1) and diluting to 1 litre, so that 1 ml. ≡ 0·25 mg. Mn.

Prepare a series of standards by adding 0 to 10 ml. of the manganese solution to solutions of the mixed nitrates contained in 100-ml. beakers, to cover the range 0 to 0·5 per cent. Mn. Add 10 ml. of nitric acid and 5 ml. of phosphoric acid to each beaker and oxidise the manganese to permanganate, following the procedure already described. Before plotting a graph relating manganese content with drum readings, correct the readings for any manganese found in the blank solution, so that the curve shall pass through the origin.

Results on commercial alloys

The methods outlined for the determination of nickel, cobalt, iron and manganese have been successfully applied to the analysis of commercial alloys. Table V gives duplicate results for the analysis of a number of these alloys and indicates the precision that the methods afford.

Table V

Analysis of commercial alloys

Sample No.	Nickel %	Cobalt %	Iron %	Manganese %	Mean total %
1.	28·81	17·37	53·44	0·18	99·81
	28·87	17·32	53·44	0·18	
2.	28·88	17·32	53·55	0·29	100·05
	28·89	17·25	53·62	0·29	
3.	29·36	16·79	53·44	0·46	99·89
	29·34	16·81	53·11	0·46	
4.	29·19	17·22	53·31	0·30	100·03
	29·22	17·19	53·33	0·30	
5.	29·27	17·09	53·34	0·39	100·19
	29·36	17·02	53·51	0·39	
6.	29·43	16·97	53·34	0·07	99·84
	29·29	16·95	53·56	0·07	
7.	29·43	17·01	53·16	0·33	100·00
	29·68	17·03	53·03	0·32	

THE IRON - COBALT - DIMETHYLGLYOXIME COMPLEX

As mentioned in an earlier part of the paper, when attempts were made to precipitate nickel with dimethylglyoxime from synthetic solutions containing nickel, iron and cobalt in proportions similar to those of the alloys with which we were concerned, brown slimy precipitates were obtained, difficult to filter and differing greatly in appearance from the characteristic bright red nickel compound. These precipitates were examined by X-ray diffraction methods. For reference, X-ray patterns were also obtained for the pure nickel dimethylglyoxime compound and for dimethylglyoxime reagent; both of these substances gave well-defined patterns and were found to be essentially crystalline.

The brown precipitates obtained initially gave X-ray patterns showing a mixture of phases of which two were predominant, one being the pure dimethylglyoxime compound, the other what was presumed to be the iron - cobalt - dimethylglyoxime complex mentioned by Weeldenburg.[3] No alteration of the lattice parameters of the nickel complex was observed.

It was found that similar brown precipitates with dimethylglyoxime could be obtained from solutions containing iron and cobalt only; the amount of the precipitate and the ease with which it formed varied according to the ratios of iron to cobalt in the solution and to the length of time of standing.

The investigation covered a range of compositions from 100 per cent. cobalt to 100 per cent. iron and the results are summarised in Table VI.

TABLE VI

Ratio of iron to cobalt in solution Fe : Co	Composition of precipitate*	Iron and cobalt in precipitate % Fe	% Co	Ratio of iron to cobalt in precipitate by weight
0 : 100	No precipitate			
10 : 90	Mainly β phase Some α			
25 : 75	Mainly β phase Some γ	5·8	9·3	0·62 : 1
48·7 : 51·3 (Equi-atomic)	Mainly β phase	8·2	10·2	0·8 : 1
75 : 25	Mixture of β and γ phases	16·2	9·5	1·7 : 1
90 : 10	Mainly "free" dimethylglyoxime			
100 : 0	No precipitate			

* Most of the precipitates contained, in addition, some "free" dimethylglyoxime in varying proportions.

The exact composition of the precipitates obtained from the various mixtures was affected to some extent by the time of standing but, in general, it was found that cobalt-rich mixtures tend to give precipitates consisting mainly of one phase, referred to in Table VI as the β phase, the material which constituted the major contaminant of the nickel precipitates we obtained in our initial experiments. Iron-rich mixtures give precipitates consisting of a mixture of two phases, referred to in the Table as β and γ; the latter is probably richer in iron.

Most of the precipitates obtained consisted of more than one phase and, after long standing, most contained significant quantities of free dimethylglyoxime. It was not possible, therefore, to determine the exact composition of the various phases in terms of iron - cobalt - dimethylglyoxime ratios, although it was established that the iron-to-cobalt ratio varied considerably according to the composition of the reacting solution.

X-ray examination of the precipitate obtained from the equi-atomic iron - cobalt mixture, however, gave a single β phase, contaminated only with free dimethylglyoxime.

According to Weeldenburg, the compound containing 1 atom of iron, 1 atom of cobalt and 3 molecules of dimethylglyoxime, to which he ascribed the formula $FeCoC_{12}H_{19}N_6O_6$, is obtained by precipitation from solutions containing iron and cobalt in equi-atomic proportions and precipitation is said to be quantitative. Because of the presence of free dimethylglyoxime in the precipitate it was not possible to determine the ratio of metal to oxime, but the ratio of iron to cobalt was found to be 0·8 : 1.

While our results, in the main, confirm the observations of Weeldenburg, Brunck, and Balz, they amplify them in the sense that the nature and degree of contamination of the nickel complex when precipitated from mixtures containing iron and cobalt is determined by the relative proportions of iron and cobalt in the mixture. Our results differ from theirs in that no constant ratio of iron to cobalt has been found in any of the iron - cobalt complexes, even from solutions containing the metals in equi-atomic ratio.

The substance that we have called the β phase, obtained either alone or in admixture over the greater part of the range of iron - cobalt mixtures, probably corresponds to the compound reported by Weeldenburg. Our results suggest that while it is possible that the ratio of metal atoms to molecules of dimethylglyoxime in the β phase obtained from solutions over a range of compositions remains constant at 2 : 3, iron and cobalt may replace each other to a considerable extent without giving rise to any very obvious changes in the X-ray pattern.

It was observed that when the ratio of iron to cobalt in a solution exceeds certain limits, in either direction, the colloidal β phase gives place to a crystalline α phase in the case of high cobalt, or a colloidal γ phase in the case of high iron ratios, but the compositions of these phases are not known.

The small crystal size as shown by the X-ray patterns probably explains and accounts for the slimy nature of these iron - cobalt complexes and for the difficulty experienced in filtering them. Even slight contamination of the nickel precipitate is evidenced by a distinct change in the characteristic bright red colour of the dimethylglyoxime complex, but the iron - cobalt complex is not isomorphous with that of nickel and, provided the conditions set out in the paper are established, it would seem probable that a clean separation of nickel can be made from mixtures with iron and cobalt over a wide range of compositions.

SUMMARY

Methods are given for the accurate determination of nickel, cobalt, iron and manganese in low-expansion alloys used for glass-to-metal seals.

In the determination of nickel, interference is caused by the formation of an insoluble complex containing iron and cobalt associated with dimethylglyoxime. This can be prevented by a preliminary reduction of the iron to the ferrous state and nickel can then be precipitated in the normal way, free from iron and cobalt.

Cobalt is determined by electrometric titration with potassium ferricyanide after removal of manganese by precipitation as dioxide.

Iron is separated by a basic acetate process, reduced and titrated.

Manganese is determined absorptiometrically after oxidation to permanganate.

Duplicate analyses of a number of commercial alloys are given.

The nature and formation of the iron - cobalt - dimethylglyoxime complex are discussed in some detail.

Thanks are due to Mr. R. H. Jones of the Mond Nickel Co. for drawing our attention to the electrometric method for the determination of cobalt, and to Mr. J. T. Minster, of the M-O. Valve Co., for supplying details of the circuit.

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ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Colorimetric Determination of DDT in Milk and Fatty Materials. M. S. Schechter, M. A. Pogorelskin, and H. L. Haller (*Anal. Chem.*, 1947, **19**, 51–53)—Attention has recently been directed to the possible danger to public health from contamination of such materials as milk, butter, eggs, meat, and fats when farm animals consume feeding stuffs treated with DDT. Pharmacological investigations (Woodard *et al.*, *Science*, 1945, **102**, 177) have shown that ingested DDT accumulates as such in the fatty tissues of experimental animals and can be excreted in the milk. Some of the DDT is metabolised to bis(*p*-chlorophenyl)acetic acid (Grummit *et al.*, *J. Amer. Chem. Soc.*, 1945, **67**, 156; White and Sweeney, *U.S. Pub. Health Repts.*, 1945, **60**, 66) which is excreted in the urine (Neal *et al.*, *Ibid.*, 1946, **61**, 403; Ofner and Calvery, *J. Pharmacol.*, 1945, **85**, 363; White and Sweeney, *loc. cit.*). When high dosages of DDT are administered to goats and rats, the milk may become toxic enough to kill other animals drinking it (Telford, *Soap Sanit. Chem.*, 1945, **21**, 161; Telford and Guthrie, *Science*, 1945, **102**, 647).

Determination of DDT in food material is complicated by the large amount of fat accompanying it when it is extracted by organic solvents. Application of chromatography was not successful, and low-temperature precipitation of the fat by cooling the solution with solid carbon dioxide led to difficulties in filtration. Saponification methods have the disadvantage that DDT is converted into its dehydrochlorinated derivative, but are applicable when it is not necessary to ascertain whether the DDT is present as such or has decomposed into its dehydrochlorinated derivative. Observation of the solubility of fat and the insolubility of DDT in sulphuric acid led to the present method.

Procedure—To 100 g. of milk add an equal volume of 95 per cent. ethanol and divide the mixture equally between two 200-ml. centrifuge bottles (unless larger bottles are available). When the concentration of DDT is more than 5 p.p.m., take correspondingly smaller samples. Add 50 ml. of light petroleum (Skellysolve B, b.p. 60° to 70° C.) to each portion, cover the bottles with rubber caps, shake vigorously, and centrifuge at 2000 r.p.m. for 15 min. Pour the contents of both bottles into a 500-ml. separating funnel, and drain the lower layer in equal portions into the same bottles. Pass the upper petroleum layer through a 5-cm., tightly-packed, cottonwool plug, held in a glass Gooch crucible holder, into a 500-ml. Erlenmeyer flask with a standard ground-glass joint. Extract the contents of the bottles as before with two successive 25-ml. portions of light petroleum and a final 50-ml. portion, centrifuging for about 10 min. each time. Drain the aqueous layer into the centrifuge bottles, and pass the petroleum layer through the plug into the flask. Place a glass bead in the flask and remove the light petroleum by distillation in an all-glass apparatus, inserting a tube connected with a vacuum line to remove the last traces of solvent. Wash the residue quantitatively into a 500-ml. separating funnel with 150 ml. of chloroform.

In the analysis of butter or fat, a 5-g. sample, or an extract thereof from which the solvent has been removed, takes the place of this residue.

Place 100 ml. of chloroform in a second separating funnel and extract the chloroform solution in the first funnel successively with (i) 50 ml. of sodium sulphate and sulphuric acid mixture (100 g. of anhydrous sodium sulphate in 1 litre of conc. sulphuric acid), (ii) 50 ml. of sodium sulphate and sulphuric acid mixture, (iii) 50 ml. of fuming sulphuric acid and sulphuric acid mixture (equal volumes of fuming sulphuric acid containing 20 to 30 per cent. of sulphur trioxide and conc. sulphuric acid), and (iv) 50 ml. of sodium sulphate and sulphuric acid mixture. Repeat the last extraction if it does not yield a light-coloured extract. Drain each acid washing from the first funnel into the second funnel and finally into a 250-ml. cylinder. The extraction in the second funnel minimises loss of DDT by slight emulsification of chloroform in the acid liquids. The funnels should be shaken vigorously each time and allowed to stand for 10 to 15 min. before the acid layer is drained off. On the rare occasions when the emulsion does not break, the mixture may be centrifuged.

Filter the chloroform from the first funnel and then that from the second funnel through a 5-cm., tightly-packed, cottonwool plug into a third separating funnel. Pipette off any chloroform that has risen to the surface from the combined acid washings in the cylinder and run it through the plug of cottonwool. Rinse the two funnels and the plug with 50 to 100 ml. of chloroform. Add enough 5 per cent. sodium bicarbonate solution (40 ml.) to the combined chloroform filtrates so that the mixture will remain alkaline after vigorous shaking. After about 10 min., filter only the chloroform layer through a similar plug into a 500-ml. Erlenmeyer flask with a standard joint. Wash the sodium bicarbonate solution left in the funnel with two successive 30-ml. portions of chloroform, and run these also through the plug into the flask. If the filtrate is not clear, repeat the filtration.

Introduce a glass bead into the flask and reduce the volume of the solution by removal of chloroform to about 10 ml. Wash this quantitatively into a large test tube (25 × 200 mm. or larger) with acetone, add a glass bead, and cautiously evaporate the solvent on the steam-bath, removing the last traces by means of a tube connected with a vacuum line. Nitrate the residue with 5 ml. of nitrating mixture and complete the analysis as described by Schechter *et al.* (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 704). Since there will still be some interference from the raffinate from the butter fat, it is advisable to make spectrophotometric measurements at 600, 620, and 640 mμ. and to average the results. Below 600 mμ., there may be enough interference to cause high results, so that it may be impossible to calculate the amount of *p,p'*-DDT and *o,p'*-DDT and add them to obtain the amount of DDT. It is therefore desirable to use a standard sample of the same DDT as was used in the feeding experiments, or, failing this, DDT of the same type and grade.

Biological tissues may be treated with sodium sulphate as described by Smith and Stohlman (*U.S. Pub. Health Repts.*, 1944, **59**, 984) or Ofner and Calvery (*loc. cit.*). After evaporation of the solvent used for extraction, the residue may be dissolved in chloroform and submitted to the sulphuric acid extraction described. *p,p'*-DDA [bis(*p*-chlorophenyl)acetic acid] is removed by the sulphuric acid treatment and does not interfere. If its determination is required, it should first be separated from the ether solution of the sample (or an extract thereof) with sodium bicarbonate solution, which is then separated, acidified, and extracted with fresh ether. After removal of the ether, the residue containing the *p,p'*-DDA can usually be nitrated directly and determined by comparison with *p,p'*-DDA standards by the Schechter - Haller colorimetric procedure or, with small amounts, the residue may be submitted to a modified sulphuric acid treatment in which the fuming sulphuric acid and the sodium bicarbonate washes are omitted, four sulphuric acid and sodium sulphate washes and one wash with 40 ml. of water being used.

Dehydrochlorinated DDT [1:1-dichloro-2,2-bis-(*p*-chlorophenyl)ethylene] is not removed by the sulphuric acid treatment and its presence may be detected by its effect on the absorption spectrum of the developed colour in the Schechter - Haller colorimetric test. It gives a red colour, with an absorption spectrum different from that of the blue colour given by DDT. *o,p'*-DDT and its dehydrochlorinated derivative would likewise be unaffected by the sulphuric acid treatment.

Recovery experiments with milk containing known amounts of DDT were satisfactory. The blank value given by the milk used was 0·38 p.p.m. (calculated as DDT), but the characteristic blue colour did not appear. The DDT content of milk from cows fed with DDT-treated material ranged from 3 to 26 p.p.m., that of butter ranged from 456 to 534 p.p.m., that of lean steak was 4 p.p.m., and that of the fat from the steak, 178 p.p.m. Milk containing 1 p.p.m. of DDT gives an easily discernible blue colour. A. O. J.

Determination of DDT in Milk by Determination of Organic Chlorine. R. H. Carter (*Anal. Chem.*, 1947, **19**, 54)—Mix 200 ml. (or 200 g.) of well homogenised milk with 200 ml. of ethyl alcohol in a separating funnel, and shake the liquid gently with 250 ml. of a mixture of 75 per cent. of ethyl ether and 25 per cent. of light petroleum (Skellysolve B, b.p. 60° to 70° C.). Draw off the aqueous layer and repeat the extraction three times with 100-ml. portions of the solvent mixture, shaking vigorously for 5 min. Discard the aqueous phase. Transfer the combined extracts to a suitable flask, remove the solvent on the steam-bath, and remove the last traces of water by addition and distillation of two 50-ml. portions of benzene.

To the residue in the flask add 150 ml. of *iso*-propanol and about 6 g. of metallic sodium cut into small pieces. Heat under refluxing conditions for 2 hr. with moderate boiling. Add 25 ml. of ethyl alcohol, allow time for complete reaction with the remaining sodium, and dilute with 100 ml. of water poured through the condenser. Evaporate the liquid to low bulk in a beaker on the steam-bath, dilute to about 400 ml. with water, and warm if necessary to dissolve the soap. Acidify with sulphuric acid, cool with ice or water, and remove the precipitated fatty acids by filtration, washing them twice with water. Extract the combined filtrate and washings twice with 100-ml. portions of the mixed solvent (*supra*). Make the aqueous solution alkaline to phenolphthalein with 2 *N* potassium hydroxide and, if necessary, reduce the volume to about 300 ml. by evaporation. Acidify the solution with a slight excess of nitric acid and determine the chloride ion by any of the standard methods. Calculate the amount of DDT by multiplying the amount of chlorine by 2.

The recovery of known amounts of DDT added to milk was not less than 95 per cent. Determination of organic chlorine in milk from cows fed on a diet free from DDT showed less than 0·2 p.p.m. of chlorine. In milk from cows receiving DDT in the diet, the corresponding figure ranged from 1 to 12 p.p.m., and these results were confirmed by determination of DDT by the colorimetric method of Schechter *et al.* (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 704; *cf.* preceding abstract).

The method presented is simple, rapid, and reasonably sensitive, but is not specific for DDT. It is possible that other halogen-containing organic insecticides, fungicides, and herbicides, *e.g.*, Spergon (tetrachlorobenzoquinone), 2:4-dichlorophenoxyacetic acid (2 : 4-D), *p*-dichlorobenzene, and benzene hexachloride may also be absorbed and stored in milk and meat products. A. O. J.

Colorimetric Assay of Digitoxin by the Modified Raymond Method. R. C. Anderson and K. K. Chen (*J. Amer. Pharm. Assoc., Sci. Ed.*, 1946, **35**, 353–355)—A chemical estimation, based upon the method of Raymond (ANALYST, 1938, **63**, 478; 1939, **64**, 113), depending upon the reaction of *m*-dinitrobenzene with digitoxin is described and compared with the method of Bell and Krantz (*J. Pharmacol.*, 1946, **87**, 198) and with the U.S.P. biological assay on cats. The latter method is the more accurate, but a chemical method is desirable for checking intermediate manufacturing stages and the final products.

Procedure—Take 10 ml. of dilute digitoxin solution in 47·5 per cent. ethyl alcohol, add 1 ml. of a 1 per cent. solution of *m*-dinitrobenzene in absolute alcohol and place in an ice-bath. After 5 to 10 min., add 2 ml. of a 20 per cent. solution of sodium hydroxide. Mix and return the solution to the ice-bath. An indigo-blue colour is produced and is measured in a colorimeter with an orange filter, exactly 5 min. after the addition of the sodium hydroxide. The colour fades fairly rapidly. Construct a standard curve using volumes of 0·75 ml., 1 ml., and 1·25 ml. of a 0·1 per cent. solution of digitoxin, diluted to 50 ml. with 47·5 per cent. ethyl alcohol. Standards so produced correspond to concentrations of 0·015, 0·020, and 0·025 mg. of digitoxin per ml. Treat samples of each solution in the manner described above, using the colorimeter readings to construct a calibration curve.

When a series of dilutions of digitoxin were assayed by this method the results obtained were within ± 5 per cent. of the values obtained by the Bell and Krantz method, and agreed with the theoretical value to the same extent. When repeated tests were made upon the same sample of digitoxin, assayed by the U.S.P. cat method, the largest experimental errors were − 3·2 per cent. and + 2·6 per cent. with the modified Raymond method and − 4·5 per cent. to + 4·2 per cent. with the Bell and Krantz method. The relative potencies of 21 different batches of digitoxin were determined by all three methods. The maximum variations from the biological assay were, by the method described, + 12 per cent. and − 14 per cent.; and by the Bell and Krantz method, + 14 per cent. and − 11 per cent. All assay results were within the limits of tolerance of ± 20 per cent. required by U.S.P., and the extreme difference between the two colorimetric methods did not exceed 18 per cent.

Assay of tablets—Take tablets equivalent to 6 mg. of digitoxin, triturate, and transfer to a 60-ml. centrifuge tube, add 60 ml. of 80 per cent. ethyl alcohol, securely stopper the tube, and agitate mechanically for about 12 hr. Centrifuge, and then take 5 ml. of the supernatant liquid and dilute to 25 ml. with 47·5 per cent. ethyl alcohol. Develop the colour as described. This solution theoretically contains 0·02 mg. per ml. Results obtained using eleven batches of tablets in this way gave results closely in agreement by either colorimetric method. In contrast with the Bell and Krantz method, it was found that lactose used in the preparation of the tablets did not interfere with the reaction in spite of the prolonged extraction with the alcohol. Neither colorimetric method is specific for digitoxin since similar colour reactions take place with other cardiac glycosides, and therefore their principal value is in manufacturing control. The method was also applied to ampoules with satisfactory results. R. H. T.

Chemical Estimation of Nicotinic Acid. A. Mueller and S. H. Fox (*J. Biol. Chem.*, 1947, **167**, 291–292)—In the cyanogen bromide - aniline method for the estimation of nicotinic acid, organic amine is not necessary for colour formation if an ammoniacal buffer solution is used. The canary-yellow colour obtained by reaction with cyanogen bromide and ammonia has a high extinction coefficient ($E^{1\%}_{1\,cm.}$ is 4500 to 4600) with a wave-length maximum at 410 mμ. The colour reaches maximum intensity within 2 to 2·5 min., and then fades rapidly. Nicotinamide reacts more slowly and gives a less intense coloration. The wave-length maximum is at 398 mμ., reaching maximum intensity in 6 min. with a slightly lower $E^{1\%}_{1\,cm.}$ value than that of the acid.

Procedure—To 1 ml. of nicotinic acid solution, containing 1 to 15 μg., add 2 ml. of ammonia buffer (0·1 *M* ammonia, 0·5 *M* dipotassium phosphate, and 2·0 *M* ammonium chloride), followed by 5 ml. of 10 per cent. cyanogen bromide solution. Prepare a reagent blank with 1 ml. of water in place of the nicotinic acid solution, and evaluate the colour at 410 mμ. at intervals between 2 and 3 min. after addition of the reagents. Calculate the nicotinic acid content from the maximum value thus obtained. F. A. R.

Biochemical

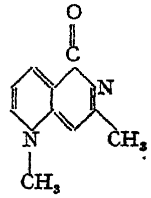
Microbiological Method for the Estimation of Nicotinic Acid based on the use of Proteus HX19. N. Grossowicz and E. Sherstinsky (*J. Biol. Chem.*, 1947, **167**, 101–105)—*Method*—Maintain cultures of *Proteus* HX19 on standard agar slants and make transfers at monthly intervals, incubating at 30° C. for 24 hr. and then storing in the refrigerator. Prepare a basal medium of the following composition: ammonium sulphate, 0·75 g.; potassium dihydrogen phosphate, 4·50 g.; potassium chloride, 0·50 g.; sodium nitrate, 1·00 g.; water, 750 ml. Adjust to *p*H 7·4 by addition of 2 to 2·5 ml. of 10 *N* sodium hydroxide, transfer 7·5-ml. quantities to test tubes, plug with cotton-wool, and autoclave at 15 lb. for 30 min. To each tube, add the following sterile solutions under aseptic conditions: 30 per cent. glucose solution, 0·2 ml.; 0·1 per cent. ferric citrate solution, 0·1 ml.; 0·5 per cent. magnesium sulphate solution, 0·1 ml.; 10 per cent. acid-hydrolyzed casein, 0·1 ml. (This is prepared as follows: To 50 g. of casein, add 250 ml. of 25 per cent. sulphuric acid, autoclave for 10 hr. at 15 lb. pressure, and remove the sulphuric acid with baryta. Carefully remove the excess of baryta by addition of sulphuric acid to *p*H 4 to 5 and then stir the filtrate with 2 per cent. of Norit for 30 min., to adsorb traces of nicotinic acid, and filter. Adsorption is complete if the organism shows only scanty growth in absence of nicotinic acid and full growth after addition of 0·15 μg. of nicotinic acid per 10 ml. of medium. Finally, adjust the hydrolysate to *p*H 7·0 with concentrated sodium hydroxide solution and dilute or concentrate to give a final concentration of 10 per cent.) Add graded amounts of a standard nicotinic acid solution containing 0·0, 0·005, 0·01, 0·02, 0·03, 0·04, 0·06, 0·08, and 0·1 μg. of nicotinic acid per 10 ml., or the solution to be tested, and make up the volume in each tube to 10 ml. If sulphonamides are present, add 0·1 ml. of a 0·1 per cent. solution of *p*-aminobenzoic acid. Suspend a loopful of *Proteus* organisms in sterile 0·9 per cent. saline to give a suspension just barely turbid, and add 1 ml. to 100 ml. of saline. Add 2 drops of the resulting suspension to each tube and incubate in a sloping position (angle, 10° to 15°) for 40 hr. at 30° C. Measure the growth of the organism in each tube turbidimetrically or by means of a photo-electric colorimeter, and calculate the nicotinic acid content of the test solution from the curve obtained with the standard solutions. Nicotinamide and nicotinic acid are equally effective, and recovery experiments gave results with an error not exceeding ± 10 per cent. Some other pyridine derivatives stimulate the growth of the organism, but other members of the vitamin B complex do not. F. A. R.

Rapid Fluorimetric Method for the Estimation of the Total Pyridine Nucleotides in the Red Blood Cells. N. Levitas, J. Robinson, F. Rosen, J. W. Huff, and W. A. Perlzweig (*J. Biol. Chem.*, 1947, **167**, 169–175)—The alkali-acetone reaction for N^1-methylnicotinamide (*cf.* preceding abstract) yields with both pyridine nucleotides a highly fluorescent product that can be used for the estimation of the co-enzymes in amounts as small as 1 μg.

Procedure—Collect 5 ml. of blood in a tube containing dry ammonium oxalate (6 mg.) and potassium oxalate (4 mg.). Transfer 2·0 ml. of the blood to a centrifuge tube containing 2·0 ml. of 25 per cent. trichloroacetic acid and 6·0 ml. of water, leave for several minutes, and then centrifuge for 5 to 10 min. at 3000 r.p.m. and filter through a No. 30 Whatman paper. Put 0·50 ml. of the filtrate into each of three fluorimeter cuvettes, A, B, and C. To A add 0·5 ml. of water; to B, 1·0 ml. of water; and to C, 0·50 μg. of N^1-methylnicotinamide in 0·50 ml. of standard solution. To tubes A and C, add 0·5 ml. of acetone, and then to each of the three tubes add 0·20 ml. of 6 *N* sodium hydroxide and mix immediately. Leave at room temperature for 5 min. and then add 0·3 ml. of 6 *N* hydrochloric acid and mix at once. Immerse the tubes in boiling water for 2 min., add 1 ml. of 20 per cent. potassium dihydrogen phosphate solution, and dilute to 10 ml. with water. Measure the fluorescence of each solution as described for N^1-methylnicotinamide, and calculate the result in a similar manner.

F. A. R.

Determination of N^1-Methylnicotinamide in Urine. J. W. Huff and W. A. Perlzweig (*J. Biol. Chem.*, 1947, **167**, 157–167)—When N^1-methylnicotinamide is treated with acetone and alkali in the cold, and then heated in acid solution, a highly fluorescent and stable compound is formed. This has been identified (Huff, *J. Biol. Chem.*, 1947, **167**, 151) as 1: 7-dimethyl-5-oxo-(1: 5-dihydro-1: 6-naphthyridine) hydrochloride:



On the formation of this compound has been based a much more sensitive and simple method of estimating N^1-methylnicotinamide in urine than any previously described.

Procedure—Put 2·00 ml. of the urine and 0·4 ml. of glacial acetic acid into each of two 25-ml. graduated cylinders, and to one of them add 1 ml. of a standard solution containing 10 μg. of N^1-methylnicotinamide. Dilute the contents of both cylinders to 20 ml. (or a volume such that the test solution contains 0·5 to 1·2 μg. of N^1-methylnicotinamide per ml. and 2 per cent. acetic acid) with water. To each cylinder add 0·1 g. of charcoal, shake, and filter immediately through a No. 30 Whatman paper. Put 1·00 ml. of the diluted urine filtrate into each of two cuvettes, A and B, and 1·00 ml. of the diluted urine filtrate containing the standard into another cuvette, C. Add to A and C, 0·5 ml. of acetone (freed from fluorescent substances by distillation over potassium permanganate), and to B, 0·5 ml. of water, mix each, and then add to each tube 0·20 ml. of 6 *N* sodium hydroxide, mix immediately, and leave at room temperature for 5 min. To each tube add 0·3 ml. of 6 N hydrochloric acid, and mix at once. Immerse in boiling water for 2 min., cool, and add 1 ml. of 20 per cent. w/v potassium dihydrogen phosphate solution and dilute with water to a volume of 10 ml. Adjust the galvanometer reading of the fluorimeter to 100 with a solution containing 0·3 μg. of quinine sulphate per ml., and then measure the fluorescence of tubes A, B, and C. Then, if A, B, and C are the readings obtained with these tubes, and if the acidified urine was diluted to 20 ml. (see above), the amount of N^1-methylnicotinamide in milligrams in the total volume of urine

$$= \frac{A - B}{2(C - A)} \times \frac{\text{vol. of urine, ml.}}{0{\cdot}1}$$

The normal excretion by an adult on an adequate diet ranged from 3 to 17 mg., with an average value of 7 mg. per day. F. A. R.

Application of Sendroy's Iodimetric Chloride Titration to Protein-containing Fluids. D. D. Van Slyke and A. Hiller (*J. Biol. Chem.*, 1947, **167**, 107–128)—*Method*—Put 1 ml. of plasma or serum into a 50-ml. centrifuge tube and add 25 ml. of phosphoric and tungstic acid reagent (dissolve 6 g. of $Na_2WO_4.2H_2O$, reagent grade, containing less than 0·01 per cent. of chloride, in 1 litre of 0·15 M phosphoric acid; if chloride-free tungstate is not available, substitute 2 g. of picric acid). Add about 0·3 g. of silver iodate and shake *vigorously* for 40 sec. During this period, proteins are precipitated and the reaction between silver iodate and chloride ion is completed: $AgIO_3 + Cl' = IO_3' + AgCl$. Centrifuge for 1 min. at 2500 r.p.m., and transfer a 10-ml. portion of the supernatant liquid to a 50-ml. conical flask. Add 1 g. of sodium iodide and titrate the liberated iodine with 0·02303 N sodium thiosulphate solution, using 2 drops of 1 per cent. starch solution as indicator. Calculate the amount of sodium chloride in grams per litre by multiplying the titre by the factor 0·5845.

The method can also be applied to Folin - Wu or Somogyi filtrates. To 1 volume of 1 : 10 filtrate, add 1·5 volume of 0·15 M phosphoric acid, shake with 0·3 g. of silver iodate per 10 ml. of filtrate, centrifuge, and titrate as described above. In the analysis of whole blood, the phosphoric and picric acid reagent should be used (dissolve 5 g. of picric acid in 1 litre of water and 10 ml. of phosphoric acid). The analysis of urine or gastric juice is carried out exactly as described for plasma, except when less than 50 mg.-mols. of chloride per litre are present. A sample 5 times that normally employed is then used, the volumes of reagent being kept the same. Milk is analysed in the same way as low-chloride urine. F. A. R.

Organic

Isolation and Identification of Fatty Acids as bis-(p-dimethylaminophenyl)-ureides. F. L. Breusch and E. Ulusoy (*Arch. Biochem.*, 1946, **11**, 489–498)—Free fatty acids are isolated and characterised as the crystalline ureides of bis-(*p*-dimethylaminophenyl)-urea; the derivatives are formed directly by boiling a solution of the fatty acid with a solution of the urea derivative in ether. Most of the ureides are only slightly soluble in ether, and crystallise directly from the ether solution even in the presence of large quantities of neutral fat.

Preparation of the reagent—The bis-(*p*-dimethylaminophenyl)-urea was prepared via dimethylaniline → *p*-nitrosodimethylaniline → *p*-aminodimethylaniline (+ carbon disulphide) → bis-(*p*-dimethylaminophenyl)-thiourea → free base (Zetsche and Röttger, *Ber.*, 1939, **72**, 1599, 2095). The free base forms white crystals, m.p. 86° to 89° C., and is soluble at 21° C. in ether (6·5 per cent.), acetone (14·2 per cent.), alcohol (0·6 per cent.), and acid-free ethyl acetate (12·1 per cent.); it is insoluble in water, but readily soluble in acids.

Preparation of ureides—Boil under refluxing conditions for 2 to 3 hr. a 1 to 4 per cent. solution of the fatty acid in ether with approximately the necessary molar amount of a saturated, clear solution of the base in ether. The ureides separate chiefly during boiling, but sometimes after cooling in an ice-box or after partial evaporation of the ether. The derivatives are always the mono-ureides, containing one mol. of acid and one mol. of base. The yield is about 80 per cent.; the presence of free water decreases the yield. The ureides are readily soluble in 5 per cent. hydrochloric acid, but not in the 0·1 per cent. acid; they can be extracted quantitatively from ether solutions by means of the 5 per cent. acid, and by precipitation with cold alkali the crystalline ureide is regained unchanged. Attempts to titrate them in alcohol-benzene solution with hydrochloric acid failed.

Direct separation of fatty acids from neutral lipids—Application to propionic acid mixed with triolein—Three hundred mg. of propionic acid, 5·0 g. of pure triolein, and 1 g. of the free base were heated in 40 ml. of ether for 2 hr. under refluxing conditions. After cooling for 2 hours in an ice-box, 1·11 g. of crystalline propionic acid ureide (m.p. 155° to 158° C.) could be isolated by filtration. After one recrystalisation from acetone, the m.p. was 161° C. Excess of free base could readily be eliminated from the ether solution by shaking with dilute hydrochloric acid, leaving a practically pure ether solution of unaltered triglyceride.

Separation of ureides from lipids—As traces of a formed ureide, unprecipitated because of its solubility in ether may in some cases remain in a lipoid solution, extraction with dilute hydrochloric acid was used to remove practically all ureide. For example, a solution of 250 mg. of pure stearic acid ureide (m.p. 122° C.) in 100 ml. of pure ether was extracted 4 times with 50-ml. portions of 5 per cent. hydrochloric acid. After separation, the evaporated ether layer was found to contain only 5 mg. of crystals, showing that 98 per cent. of the ureide had been removed. The stearic acid ureide could be precipitated unaltered (m.p. 121° C.) from the clear solution in hydrochloric acid by addition of alkali.

The properties of the bis-(*p*-dimethylaminophenyl)-ureides of the normal fatty acids from C_1 to C_{20} (except C_{13}, C_{15}, C_{17}, and C_{19}); of the α, β-unsaturated fatty acids crotonic, β-methylcrotonic, 1-pentenoic, 1-nonenoic, 1-decenoic, 1-hendecenoic, 1-dodecenoic, 1-tridecenoic, and 1-tetradecenoic; of the unsaturated acids 10-hendecylenic, oleic, stearolic, ricinoleic, 9 : 11-linoleic, 9 : 12-linoleic, and 9 : 12 : 15-linolenic; and of racemic mixtures of the *d*- and *l*-acids of β-hydroxypelargonic, β-hydroxycapric, β-hydroxyhendecylic, β-hydroxylauric, and β-hydroxytridecanoic acids, are tabulated. E. M. P.

New Specific Colour Reaction of Hexuronic Acids. Z. Dische (*J. Biol. Chem.*, 1947, **167**, 189–198)—*Method*—Mix, with cooling, 1 ml. of the solution, containing 5 to 100 μg. of uronic acid, with 6 ml. of concentrated sulphuric acid and heat for 20 min. in boiling water. Cool to room temperature and add 0·2 ml. of a 0·1 per cent. alcoholic solution of pure carbazole with shaking. After a few minutes, a pink colour appears; the intensity increases for 2 hr. and remains practically unchanged for at least an hour. Later, the colour slowly decreases and loses about one-third of its intensity after 24 hr. The pink colour is sensitive to water and, if 3·8 ml. of water are added, with cooling, to the reaction mixture, the colour fades rapidly and disappears completely in about 3 min. If the mixture is left at room temperature for many hours, a weak violet colour appears; this reaction is common to all sugars. The formation of the pink colour, however, is characteristic of uronic acids, true sugars giving no visible reaction at dilutions of the order of 0·01 per cent. The reaction is also negative with ascorbic, mucic, hydroxypyruvic, 2-keto hexonic, pyruvic, acetoacetic, lactic, fumaric, succinic, malic, acetic or formic acid, acetone, urea, or guanidine. Positive reactions are given by conjugated acids and polyuronides, and the intensity corresponds approximately to their hexuronic acid contents. F. A. R.

Determination of Dipentaerythritol in Mixtures of Pentaerythritol and Dipentaerythritol. J. A. Wyler (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 777–778)—The method is based on the crystallisation of dipentaerythritol from aqueous solutions containing an approximately 1 : 1 mixture of di- and mono-pentaerythritols. This ratio is obtained, if necessary, by adding pure dipentaerythritol to the solution of the sample before analysis. The precipitated dipentaerythritol is weighed directly, and corrections are applied for the quantity of dipentaerythritol added and that remaining in solution.

Procedure for the analysis of a mixture of 80 *parts of pentaerythritol and* 20 *parts of dipentaerythritol*—Weigh exactly 2·500 g. of the sample and 2·500 g. of pure dipentaerythritol in a tared aluminium dish, and transfer to a 125-ml. Erlenmeyer flask weighed to the nearest 0·1 g. Add exactly 60 g. of distilled water, shake gently, and heat on an electric hot-plate to complete solution; prevent undue loss of water by placing a clean rubber stopper in the neck of the flask and loosening a few times to relieve pressure. Continue heating and shaking for 3 to 5 min. Weigh the flask and, if necessary, add water drop by drop to ensure the presence of 60 g. of water. Allow the flask to stand at room temperature for half an hour, with occasional gentle shaking, and then leave it overnight in a water-bath at 18° C. (not below 15° C.). Check the weight and, if necessary, add a few drops of water to ensure that the weight of water present is 60 ± 0·1 g. Place the flask in a water-bath at 25° C. and shake gently. Remove the stopper and stir the separated crystals of dipentaerythritol, keeping the flask at 25° C. for at least 10 min. Transfer the crystals to a weighed, 15-ml. Gooch-type fritted-glass crucible of medium porosity, effecting the transfer by means of exactly 40 ml. of denatured alcohol applied from a small wash-bottle. Wash the crystals with 10 ml. of ethyl ether and dry at 110° C. for 1 to 2 hr., or to constant weight.

Percentage of dipentaerythritol

$$= 100\,(A - 2{\cdot}500 + B)/2{\cdot}500,$$

where A = weight of dry crystals obtained and B is the solubility correction for the dipentaerythritol remaining in the filtrate and the washings; for the conditions specified this correction is 0·145 g.

The added dipentaerythritol must be of high purity, and the conditions followed accurately. It may sometimes be advisable to run a blank determination, a known mixture of about the same composition as the unknown being used, to test the solubility correction. The precipitated crystals should not be allowed to cake to a hard mass, but should be present as relatively fine, individual plates readily removable by means of a stirring rod and a stream of alcohol from a wash-bottle. The sample taken for analysis should be representative of the mixture under examination; dipentaerythritol has a very low packing density and a pronounced tendency to segregate when mixed with the denser and more compact pentaerythritol crystals. E. M. P.

Identification of Acidic Oxycellulose by means of Aromatic Amines and Diamines. Matya Krajčinović and Mladen Krajčinović (*J. Text. Inst.*, 1947, **38**, T11–T12)—The test depends on the formation of additive compounds between oxycellulose and aromatic amines or diamines, the additive compounds being identified by introduction of an azo group as chromophore in the benzene radicle and development of a dye. With the diamines, the amino group not combined with the carboxyl group of the oxycellulose was diazotised and the product was coupled to the oxycellulose with β-naphthol or other developer. The monoamines used were aniline, dimethylaniline, and α-naphthylamine, and the dyes were developed with diazotised *p*-nitraniline, *m*-nitraniline, and α-naphthylamine. As diamines there were used benzidine, *p*-phenylenediamine, *m*-toluylenediamine, and chloro-*m*-phenylenediamine, the coupling agents being β-naphthol, the α-naphthalide of 2 : 3-oxynaphthoic acid, Naphthol AS-BO, resorcinol, ethyl-β-naphthylamine, and phenylmethylpyrazolone.

	Developer used as coupling component				
Diamine giving the diazo-component	β-Naphthol	Naphthol AS-BO	Phenylmethylpyrazolone	Resorcinol	Ethyl-β-naphthylamine
Benzidine	reddish-violet	pale violet	orange-yellowish	yellowish-brown	pale pink
p-Phenylenediamine ..	pale reddish-violet	pale violet	orange	brownish-yellow	pale pink
m-Toluylenediamine ..	pale reddish-violet	pale violet	orange-yellowish	brownish-yellow	pale brown
Chloro-*m*-phenylenediamine	pink	violet-pink	orange-brown	pale brown	faintly pink

Method, using benzidine and β-naphthol. Moisten about 0·5 g. of fibre with 2 ml. of 0·1 *M* alcoholic benzidine solution and rinse well in water after 1 or 2 min. Diazotise by applying 15 ml. of 3 per cent. hydrochloric acid and 5 ml. of 3 per cent. sodium nitrite solution, and rinse well after 2 or 3 min. Develop the colour by adding a few drops of 0·1 *M* β-naphthol in alkaline solution, and wash the sample. The alcoholic benzidine solution must be neutral before testing, and the fibre must be treated with 0·5 per cent. hydrochloric acid for 30 min. and washed until neutral.

The above table shows the results given with diamines after diazotisation and coupling with the developers indicated. E. M. P.

Cellulose in Wood. T.A.P.P.I. Tentative Standard T17 m–46. Anon. (*Paper Trade J.*, 1946, **123**, Dec. 26th, *T.A.P.P.I. Sect.*, 217–218)—Weigh accurately approximately 2 g. of air-dry, disintegrated wood of known moisture content, in a tared, 40-ml. alundum crucible (porosity, R.A.98) contained in a weighing bottle. Extract for at least 6 hr. in a Soxhlet apparatus with a mixture of 33 volumes of 95 per cent. ethyl alcohol and 67 volumes of C.P. benzene, evaporate off the solvent, and wash the residue well with hot water, using suction. Transfer the residue to a 40-ml., glass crucible with a fritted base (porosity, Pyrex M), from the base and top of which glass tubes lead, through 3-way taps (connecting with the air), to a Hempel gas pipette and to a Hempel precision gas burette, respectively. The crucible and gas burette are water-jacketed at 23·5° to 32° C., and the burette and pipette should contain saturated calcium chloride solution, saturated with chlorine at room temperature. Remove surplus moisture from and distribute residual moisture throughout the sample by suction, first at the base and then at the top of the crucible. Pass approximately 230 ml. of chlorine, from the burette, up through the sample and into the pipette; this first chlorination should be completed in 3 to 4 min. Remove the crucible, wash the contents successively with 50 ml. each of water, approximately 3 per cent. sulphur dioxide solution, water, and freshly prepared 2 per cent. sodium sulphite solution, and transfer them to a 250-ml. Pyrex beaker by means of a pointed glass rod. Rinse out the last traces from the crucible, using, firstly four 15-ml. portions of the sulphite solution; then place 10 ml. of the solution in a watch glass and draw it into the crucible by suction from the top, repeating this operation three times. Place the beaker in boiling water for 30 min., replace the fibres in the glass crucible and wash them with 250 ml. of water. Repeat the above chlorination and extraction operations until only a faint pink colour results on adding the sulphite solution, taking only 2 to 3 min. for the chlorination stages, to avoid hydrolysis of the cellulose. Finally, wash the lignin-free wood, in the alundum crucible, successively with 500 ml. of hot water and 50 ml. each of 95 per cent. alcohol and ether, dry it at 100° to 105° C., and weigh it with the crucible, in the stoppered weighing bottle, to 1 decimal place; report the result as a percentage of the moisture-free, unextracted wood. J. G.

Qualitative Analysis of Mixtures of Cyanamide Derivatives. A. A. Korinfski (*Zavod. Lab.*, 1945, **11**, 816–821)—Cyanamide, dicyandiamide, melamine, urea, thiourea, guanidine, biguanide (diguanidine) $C_2H_7N_5$, dicyanodiamidine (guanylurea), amidodicyanic acid (cyanourea) $C_2H_3ON_3$, ammeline $C_3H_5ON_5$, ammelide $C_3H_4O_2N_4$, sulphides, and thiocyanates may be detected in complex mixtures by the following methods.

Cyanamide—To 5 to 10 ml. of a solution neutralised to litmus with dilute sulphuric acid add cadmium hydroxide in excess, shake, and leave for 3 hr.; filter 2 or 3 ml. of the solution and add 5 or 6 drops of ammoniacal silver nitrate solution to give a yellow precipitate if cyanamide is present. In absence of sulphides or thiourea the cadmium hydroxide precipitation is unnecessary. If very small amounts of cyanamide are to be detected in presence of large amounts of thiourea add, instead of ammoniacal silver nitrate, a few drops of aqueous ammonia and some lead hydroxide. In presence of cyanamide the lead hydroxide assumes a yellow colour. Cadmium hydroxide is prepared by precipitating cadmium acetate solution with sodium hydroxide and washing the precipitate with hot water until the washings give no colour with phenolphthalein. Lead hydroxide is prepared by precipitation with aqueous ammonia.

Urea—The urease reaction should be used; other methods are not sufficiently selective.

Thiourea—Acidify 3 to 4 ml. of the solution with acetic acid, add 0·5 ml. of 5 per cent. cadmium acetate solution, shake, and filter. To the filtrate add sodium hydroxide to precipitate cadmium hydroxide, and boil. A yellow coloration of the hydroxide indicates thiourea (minimum amount detectable 0·02 mg., limiting concentration 1 in 150,000).

Dicyandiamide, $H_2N.C(:NH).NHCN$—The test is based on the formation of crystalline silver monocyanoguanidine picrate (*cf.* Harger, *Ind. Eng. Chem.*, 1920, **12**, 1107; Gelhaar, *Tekn. Tidsk.*, 1933, 23). Acidify 1 or 2 ml. of the solution with acetic acid, add an equal volume of a saturated aqueous solution of picric acid and a few drops (an excess must be used) of 5 per cent. silver nitrate solution, shake, leave undisturbed for 10 to 20 min., and then transfer a drop of the solution, together with the precipitate, to a microscope slide. In presence of dicyandiamide the crystals are in the form of oblong tablets (for sensitivity, see Table).

Melamine—The picrate crystallises from neutral or acetic acid solution as a mass of entangled fibres (rapid crystallisation) or as very fine and long yellow needles (slow crystallisation from warm solution), which may be distinguished under the microscope from the picrates of ammeline and dicyanodiamidine. Melamine picrate alone may be obtained by treating 2 ml. of the solution under test with 2 ml. of glacial acetic acid and 4 ml. of saturated picric acid solution, and then shaking and leaving for 30 min. (see Table).

Ammeline—From slightly acid solution the picrate forms fat, yellow needles. Since ammeline is only slightly soluble in cold water the mixture under test should be extracted with hot water or dilute sodium hydroxide solution. As potassium picrate would precipitate later on, potassium hydroxide should not be used.

Ammelide—In absence of ammeline, treat with 2 per cent. sodium hydroxide solution, filter, and neutralise the filtrate with acetic acid, to give a white amorphous precipitate of ammelide (minimum amount detectable 0·1 mg., limiting concentration 1 in 2500). If congo red indicator is added before neutralisation the ammelide on precipitation adsorbs the indicator and becomes dark red while the solution is decolorised. In presence of ammeline, add to the solution (2 to 3 ml.) of the sample in sodium hydroxide 3 ml. of saturated picric acid solution, heat to boiling, add acetic acid until it gives a faint odour, again heat to boiling, shake, and leave for 30 min. Ammeline picrate and amorphous ammelide are easily distinguished under the microscope.

Guanidine—Add a few drops of aqueous ammonia (avoid an excess since ammonium picrate may precipitate later) to 2 to 3 ml. of the neutral solution of the sample and then an equal volume of saturated picric acid solution, shake, and leave for 1 hr. Guanidine picrate gives fine oblique plates (rapid crystallisation), or long columns (slow cooling).

Biguanide—Garby's reaction with nickel salts (*Ind. Eng. Chem.*, 1926, 819) is used.

Dicyanodiamidine, $H_2N.C(:NH).NH.CO.NH_2$—The picrate from dilute acetic acid or neutral solution consists of small rounded polyhedra. In presence of large amounts of melamine, it is best to use Grossmann's reaction (*Ber.*, 1906, **39**, 3357).

Amidodicyanic acid, $H_2N.CO.NHCN$—In dilute acetic acid solution (*p*H 4) a colourless, amorphous precipitate of $AgC_2H_2ON_3$ is obtained with silver nitrate.

Thiocyanates, by ferric chloride; *and sulphides*, by cadmium acetate.

Mixtures—With a mixture containing all these substances take 2 to 3 g. of the powdered material, shake for 30 min. with 100 to 150 ml. of water, filter, and wash 2 or 3 times with small amounts of water; then treat the filter and insoluble matter with 5 to 6 ml. of 2 per cent. sodium hydroxide solution, filter, and test the filtrate for ammeline and ammelide. On separate portions of the aqueous extract carry out tests for urea, thiocyanate, ammonium, and cyanamide (cadmium hydroxide - lead hydroxide method) and then apply the following scheme to 25 to 30 ml. of the solution—Neutralise and add 1 ml. of acetic acid and 2 ml. of 5 per cent. cadmium acetate solution (a precipitate of cadmium sulphide indicates sulphide); to the filtrate add sodium hydroxide dropwise to give a white precipitate, heat to 75° to 80° C. and leave for 30 min. (yellow colour of precipitate indicates thiourea); filter, and to the filtrate add an equal volume of saturated picric acid solution and aqueous ammonia (to faint odour), examine a drop containing precipitate under the microscope (guanidine and biguanide indicated); filter, and add to the filtrate 25 ml. of 5 per cent. nitron in 5 per cent. acetic acid (the precipitate is nitron picrate and nitron thiocyanate); filter, and add 10 per cent. ammonium nitrate solution to precipitate nitron completely, filter, and add to the filtrate 5 ml. of 5 per cent. silver nitrate solution and exactly neutralise to methyl orange (a precipitate indicates amidodicyanic acid); filter, and add to the filtrate 1 ml. of nitric acid [concentration not stated], 5 ml. of 5 per cent. silver nitrate solution, and an equal volume of saturated picric acid solution (a precipitate indicates dicyandiamide).

SOLUBILITIES OF PICRATES IN G. PER LITRE AT 20° C. AND (IN BRACKETS) LIMITING CONCENTRATIONS

Substance	Solution A	Solution B	Solution C	Solution D
Melamine	< 0·0005 (1 : 250,000)	> 0·4000 (no ppt.)	0·0006 (1 : 200,000)	0·0024 (1 : 50,000)
Ammeline	0·0039 (1 : 40,000)	0·2032 (∼ 1 : 600)	0·0034 (1 : 45,000)	0·0160 (1 : 10,000)
Dicyanodiamidine	0·0048 (1 : 30,000)	0·3148 (no ppt.)	0·0060 (1 : 25,000)	> 0·2000 (no ppt.)
Guanidine	— (1 : 4000)	— (1 : 7000)	— (no ppt.)	— (no ppt.)
Dicyandiamide*	0·0012 (1 : 80,000)	0·2264 (1 : 1000)	0·0012 (1 : 150,000)	not determined do.

* The solubilities refer to silver monocyanoguanidine picrate, the limiting concentrations to dicyandiamide.

The Table shows the solubilities at 20° C. of the picrates of melamine, ammeline, dicyanodiamidine, and silver monocyanoguanidine, and the limiting concentrations detectable through the picrates, in half-saturated aqueous picric acid (A), in half-saturated aqueous picric acid containing 1 per cent. of NH_4 (B), in half-saturated aqueous picric acid containing 5 per cent. acetic acid (C), and in half-saturated aqueous picric acid containing 25 per cent. acetic acid (D). G. S. S.

Determination of Certain Cyanamide Derivatives in Complex Mixtures. A. A. Korinfski (*Zavod. Lab.*, 1946, **12**, 418–421)—

Melamine—Precipitation of the picrate in 5 per cent. acetic acid (Zavarov, *J. Chem. Ind. Russ.*, 1945, part 2 (?)) is unsatisfactory for determining melamine in presence of large amounts of ammeline and dicyanodiamidine. In 25 per cent. acetic acid, however, dicyanodiamidine picrate does not precipitate at all, whilst ammeline picrate may be washed from the precipitate (its solubility is 0·016 g. per 100 ml.), but a small correction has to be made for the solubility of melamine picrate. The method may be used in the presence of ammelide, guanidine, dicyandiamide, and urea. *Procedure*—Mix 0·2 to 0·3 g., or 0·5 g. if the melamine content is less than 30 per cent., of the finely ground powder with 100 ml. of water by stirring with a glass rod for 20 min., filter into a 250-ml. graduated flask through a small filter, wash the insoluble matter on to the filter by means of a small quantity of water, and then wash the filter twice with cold water (this minimises the amount of ammeline in solution). Make up to the mark, mix, take 50 ml., add 50 ml. of glacial acetic acid, heat to 80° C., add 100 ml. of a saturated aqueous solution of picric acid, and leave for 2 hr. in the cold with occasional shaking. Filter by suction through a glass filtering crucible (No. 2), use the filtrate to wash the precipitate on to the filter, then wash four times with a saturated aqueous solution of melamine picrate and three times with ether. Dry for 2 hr. at 100° to 105° C., cool, and weigh. Calculate the melamine percentage from the formula, $(a + 0{\cdot}0048) \times 0{\cdot}355 \times 500/w$, where a is the weight of precipitate and w the weight of sample taken.

Ammeline—The determination of ammeline as picrate in presence of melamine and dicyanodiamidine is based on the low solubility of ammeline in water. *Procedure*—Add 100 ml. of water to a sample containing from 0·1 to 0·15 g. of ammeline in a 250-ml. conical flask, stopper, and shake for 30 min. Filter and wash two or three times with small portions of cold water, place the filter paper and insoluble matter in a 250-ml. graduated flask, add 15 ml. of 2 per cent. sodium hydroxide solution, shake for 10 to 15 min., make up to the mark, mix, and filter. Take 50 ml. of the filtrate, add 55 ml. of saturated aqueous picric acid solution, heat to boiling, add 5 ml. of glacial acetic acid, and leave at room temperature for not less than 8 hr. Filter by suction through a weighed, glass filtering crucible (No. 2), use the filtrate to transfer the precipitate, wash with saturated ammeline picrate solution, and dry the residue at 90° C. for 2 hr. Ammeline, per cent., $= (a + 0{\cdot}0037) \times 0{\cdot}3395 \times 500/w$, where a is the weight of picrate, 0·0037 a correction for solubility, and w the sample weight. The method is unsuitable in presence of ammelide.

Thiourea—in presence of cyanâmide and its derivatives, of sulphides, and of thiocyanates (*cf. Idem., ibid.*, 1945, **11**, 816). *Procedure*—Place a sample containing 0·1 to 0·2 g. of thiourea in a 250-ml. graduated flask, dissolve it by shaking with 150 ml. of water, acidify with acetic acid, add 10 ml. of 10 per cent. cadmium acetate solution to remove sulphide ions, make up to the mark, mix, filter off 50 ml., add to it 50 ml. of water, and then gradually 20 per cent. sodium hydroxide solution to precipitate cadmium hydroxide and to give an excess of 1 to 2 ml. Heat to 80° C., stirring with a glass rod, set aside to cool for 1 hr., acidify with acetic acid, filter through an ashless paper, and wash five or six times with water. Place the filter and precipitate in a beaker, break up the paper by means of a glass rod, add 50 ml. of 0·1 *N* iodine solution and 5 ml. of concentrated hydrochloric acid, and titrate with 0·1 *N* sodium thiosulphate, using starch as indicator. One ml. of 0·1 *N* iodine is equivalent to 0·0038 g. of thiourea.

Dicyandiamide, $H_2N.C(:NH).NHCN$—(i) If thiocyanates are present, dissolve a sample containing 0·1 to 0·2 g. of dicyandiamide in 100 ml. of water, acidify with acetic acid, add 10 ml. of a 5 per cent. solution of nitron in acetic acid, filter into a 500-ml. graduated flask, wash the filter four times with water, treat the filtrate with 15 ml. of 10 per cent. ammonium nitrate solution, shake, make up to the mark with water, mix, and filter. Take 100 ml. of the filtrate and determine dicyandiamide by the volumetric picric acid - silver nitrate method (the precipitated complex silver picrate is dissolved in nitric acid and the silver is determined by titration as in the U.S.S.R. Specification 10175—39). (ii) If sulphides and thiourea are present, dissolve a sample containing 0·1 to 0·2 g. of dicyandiamide in 100 ml. of water in a 250-ml. graduated flask, exactly neutralise the solution to methyl red by means of acetic acid and sodium hydroxide, add 20 to 25 ml. of cadmium hydroxide emulsion (Korinfski, *Ibid.*, 1945, **11**, 816), heat to 80° C., allow to stand for 1 hr., make up to the mark, mix, and filter. Carry out the volumetric determination on 50 ml. of the filtrate. G. S. S.

Determination of Hydroperoxides in Rubber and Synthetic Polymers. H. A. Laitinen and J. S. Nelson (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 422–425)—The method is intended for the determination of relative quantities of hydroperoxides in natural rubber and various synthetic polymers and co-polymers, and is based on the reaction between the hydroperoxide and ferrous iron in benzene - methanol solution, the excess ferrous iron being determined colorimetrically by means of *o*-phenanthroline as reagent. Interference by antioxidants is prevented by the presence of phosphoric acid.

Reagents—*Ferrous iron solution*—Remove the dissolved oxygen from 1 litre of methanol by bubbling nitrogen through it for 30 min. Dissolve 19·605 g. of ferrous ammonium sulphate hexahydrate in the methanol containing 13·9 ml. of concentrated sulphuric acid to make the solution about 0·25 *M* with respect to sulphuric acid. Stored under nitrogen, this solution is stable for a long time. For use over a period of a few days, 10-ml. portions may be allowed to stand in air. Each day, prepare a 0·002 *N* solution by diluting 2 ml. of the 0·05 *N* solution to 50 ml. with methanol, but without adding sulphuric acid. *Nitric acid - methanol solution*—Add 1 ml. of concentrated nitric acid to 20 ml. of methanol slowly and with cooling. *Phosphoric acid - methanol solution*—Prepare a molar stock solution by adding 6·75 ml. of 85 per cent. phosphoric acid (14·8 *M*) to methanol, to make 100 ml. of solution. Dilute the molar solution to 0·04 *M* for use as a working solution; 0·5 ml. of 0·04 *M* solution provides 0·02 mg.-mol. of phosphoric acid. o-*Phenanthroline solution*—Dissolve 1 g. of *o*-phenanthroline monohydrate in 1 litre of thiophene-free benzene.

Analysis of unknown samples—Pipette 1 ml. of rubber solution, containing about 1 g. of polymer in 100 ml. of benzene, into a 50-ml. volumetric flask. Add about 25 ml. of thiophene-free benzene, washing any polymer from the sides of the flask. Add 1 ml. of nitric acid - methanol solution and 0·5 ml. of the 0·04 *M* phosphoric acid solution, shaking well after each addition. Add 1 ml. of 0·002 *N* ferrous iron solution, shake well, and allow to stand for at least 15 min. Add 3 to 5 ml. of *o*-phenanthroline solution and make up to 50 ml. with thiophene-free benzene. Read the transmission value at 508 mμ. with a spectrophotometer or use a photo-electric colorimeter with a filter showing maximum transmission at 500 mμ. or 510 mμ. (preferably not 520 mμ.), comparing the transmission with that of benzene. If the transmission value is between 50 and 70 per cent., calculate the active oxygen content as shown below. Otherwise, estimate the necessary adjustment in sample size and repeat the determination. With samples of entirely unknown peroxide content, vary the sample size, using 0·1, 1, and 10 ml. of polymer solution in the preliminary estimation. If a 10-ml. sample is used, or if the sample is highly coloured, use a diluted benzene solution of the polymer instead of benzene as the comparison liquid in the optical measurement.

Construction of calibration curve—Using a graduated 1-ml. pipette, add 0·2, 0·4, 0·6, 0·8, and 1·0 ml. of 0·002 N ferrous iron solution to a series of 50-ml. volumetric flasks. Add about 25 ml. of benzene to each flask, washing the walls carefully. Form the ferrous iron complex by adding 3 to 5 ml. of the *o*-phenanthroline solution and make up to 50 ml. with benzene. Take colorimeter or spectrophotometer readings relative to benzene. Compute the optical density of each solution from the equation $D = \log I_0/I$, and construct a graph of optical density against concentration of iron, expressed as equivalents of ferrous iron per 50 ml. A straight line, corresponding to Beer's law, should be obtained.

Calculations—From the calibration curve read the amount of iron remaining unoxidised and determine by difference the amount of iron oxidised. Each g.-equivalent of iron corresponds to one g.-equivalent or 8 g. of active oxygen. Calculate the active oxygen as p.p.m. of solid polymer. If the polymer is only partly soluble, determine the concentration of the soluble polymer by evaporating an aliquot of the solution, and base the calculation on the soluble part only. $(A - B) \times 8 \times 10^6/C \times V =$ micrograms of active oxygen per g. of polymer = p.p.m. of active oxygen, where $A =$ g.-equivalents of ferrous iron taken; $B =$ g.-equivalents of ferrous iron remaining; $C =$ concentration of polymer in g. per ml. of benzene; $V =$ ml. of polymer solution used.

The results are reproducible to about 20 per cent. on a given rubber solution, but sampling difficulties may cause larger variations on solid samples. The method is sensitive to 10 or 20 p.p.m. of active oxygen, calculated on the solid polymer, and can be made sensitive to 1 p.p.m. by increasing the sample size and compensating for its colour by using a polymer solution instead of benzene as the comparison liquid. For results of absolute significance, standardisation by means of *tert.*-butyl hydroperoxide is recommended. The method is applicable only to hydroperoxides and can be used generally for compounds of low molecular weight as well as for high polymers. E. M. P.

Polarographic Investigations of Oxalate, Citrate, and Tartrate Complexes of Ferric and Ferrous Iron. J. J. Lingane (*J. Amer. Chem. Soc.*, 1946, **86**, 2448–2453)—The ferric ion gives rise to a polarographic reduction wave in acid tartrate, citrate, or oxalate solutions, of which the citrate medium is best for analytical purposes. Between *p*H 5 and *p*H 7 the reaction is reversible and the ferrous ion can be determined by its anodic wave.

In 0·5 M sodium citrate solution containing 0·005 per cent. of gelatin, at *p*H 6, the diffusion current constant, $i_d/(Cm^{2/3}t^{1/6})$, where i_d is the diffusion current, C the concentration of ferric, or ferrous, ion, m the mass of mercury flowing per sec., and t the drop time, is constant to within ± 1·5 per cent., and has a value of 0·93 ± 0·01 micro-amp./millimole/litre/mg.$^{2/3}$sec.$^{-1/2}$ at 25° C. for the cathodic wave of the ferric ion. The corresponding value for the anodic wave of the ferrous ion is 0·90 ± 0·01. At *p*H 6, the half-wave potential is − 0·22 v. *versus* the saturated calomel electrode.

Similar waves are obtained in 0·5 M sodium tartrate and 0·5 M sodium oxalate, under the same conditions, the values of the diffusion current constants of the cathodic ferric waves being 1·11 ± 0·03 and 1·50 ± 0·03 micro-amp./millimole/litre/mg.$^{2/3}$ sec.$^{-1/2}$, respectively.

In carrying out determinations on solutions containing the ferrous ion, it is necessary to add a measured volume of ferrous solution to a known volume of supporting electrolyte that has been freed from dissolved air by hydrogen or nitrogen, owing to the ease of oxidation of the ferrous complexes. J. G. W.

Inorganic

Micro-determination of Chlorides by means of Mercurous Iodate. K. Avaliani (*Zavod. Lab.*, 1946, **12**, 179–182)—Soluble chlorides react with solid mercurous iodate to give mercurous chloride and soluble iodate equivalent to the amount of chloride initially present in solution. After filtration, the soluble iodate may be determined by addition of potassium iodide and titration of the liberated iodine with thiosulphate.

Procedure—To 10 ml. of the solution under examination (preferably a sulphuric acid solution of *p*H 2·2) in a test tube, add from the end of a spatula about five times the theoretical quantity of powdered mercurous iodate, stopper the tube, shake for 2 min., filter through a dry paper, rejecting the first portion of the filtrate, and titrate 1 ml. with 0·01 N sodium thiosulphate after adding potassium iodide. Calculate the chloride content according to the equation

$$Hg_2(IO_3)_2 + 2NaCl = Hg_2Cl_2 + 2NaIO_3,$$

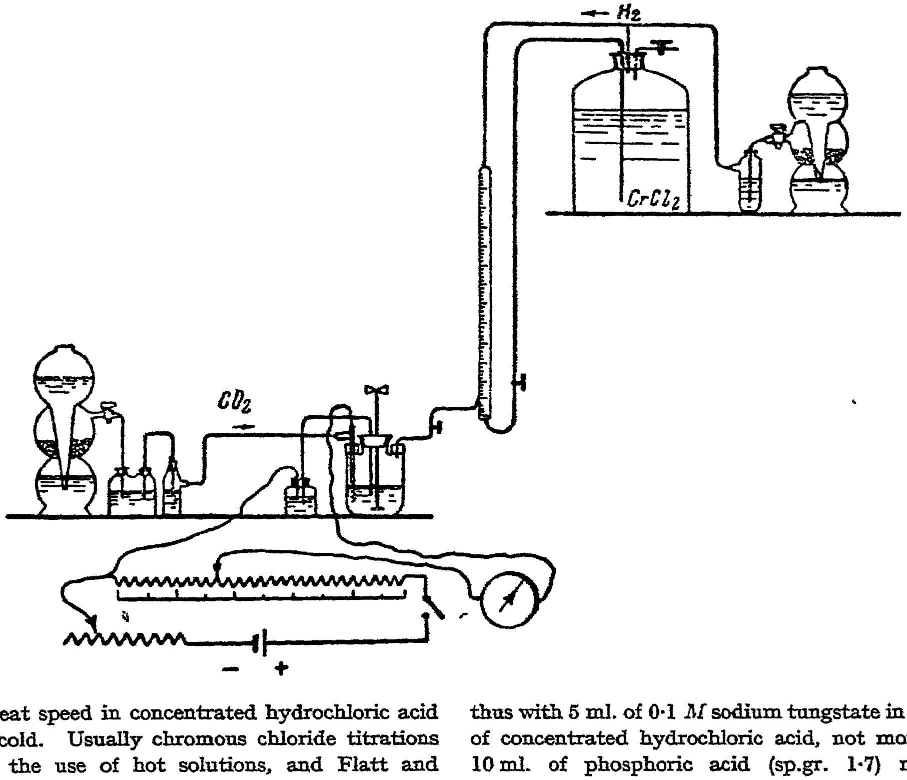
making a small correction, if necessary, as indicated below, to allow for the fact that the amount of iodate formed is a few tenths of 1 per cent. less than the theoretical.

The reagent is obtained from potassium iodate and mercurous nitrate by double decomposition. The salt is then washed with water until successive washings give the same colours with sulphuric acid and potassium iodide; it is dried over sulphuric acid and phosphorus pentoxide, and kept in a finely powdered form in the dark. The solubilities of mercurous iodate at 25° in water, and in 0·01 *N* sulphuric acid (*p*H 2·2), are too small to have any effect upon the results.

Within the range of chloride content 1 to 12 mg.-mols. per litre (or 0·058 to 0·701 g. of sodium chloride per litre), results are about 99·4 per cent. of the theoretical. G. S. S.

Bivalent Chromium Solution for the Volumetric Determination of Tungsten. Determination of Tungsten in Tungsten Concentrates and Ferro-tungsten. J. A. Tschernichov and V. G. Gorjuschina (*Zavod. Lab.*, 1946, **12**, 397–411)—The authors have shown (*Ibid.*, 1945, **11**, 137) that chromous chloride reduces sexavalent tungsten quantitatively to quinquevalent tungsten with great speed in concentrated hydrochloric acid in the cold. Usually chromous chloride titrations require the use of hot solutions, and Flatt and Sommer (*Helv. Chim. Acta*, 1944, **27**, 1518) have recently described a method for tungsten which suffers from this disadvantage. From a study of various factors influencing the reaction, details are now given of the conditions necessary for determining tungsten by means of a standard solution of chromous chloride.

Apparatus—The reservoir for the titrating solution is a 3-litre bottle of dark glass. It is fitted with a three-hole rubber bung carrying a siphon connected to the bottom of a burette, a tube connecting the space in the bottle with both a hydrogen generator and the top of the burette, and a tube with a tap. The titration vessel is a bottle of 400-ml. capacity with four necks; the central neck carries a rubber bung with a hole which is used both for the insertion of a stirrer and for outlet of the carbon dioxide which is passed, during the titration, through the solution. Through the other three necks pass (i) a carbon dioxide inlet tube and a platinum indicator electrode, (ii) a saturated potassium chloride bridge connected with a saturated calomel half-cell, and (iii) the jet of the burette. Electrical connections are made in the normal manner for potentiometric titrations (see Fig.).



Effect of complex formation—Heteropoly compounds—In presence of phosphoric acid, the potential jump at the end-point of the titration is less definite; thus with 5 ml. of 0·1 *M* sodium tungstate in 100 ml. of concentrated hydrochloric acid, not more than 10 ml. of phosphoric acid (sp.gr. 1·7) may be present. Silicic acid, even in large amounts, has no effect. *Organic acids*—Titration curves obtained with concentrated hydrochloric acid solutions of tungsten are not affected in any way by the presence of citric, tartaric, oxalic, or formic acid. In more dilute hydrochloric acid, correct results may be obtained with oxalic acid present, although the potential at the equivalence point is lower and the technique of titration is more difficult, but not with the other organic acids, owing to formation tungsten blue.

Replacement of hydrochloric acid by sulphuric acid—Without a reduction in the total acidity, clear solutions may still be obtained by using a mixture of 50 ml. of concentrated hydrochloric acid and 30 ml. of concentrated sulphuric acid in place of 100 ml. of the former acid when 5 ml. of 0·5 *M* sodium tungstate are taken. A somewhat smaller potential jump occurs at the end-point. Quantitative reduction does not occur when hydrofluoric or phosphoric acid is used instead of sulphuric acid.

Effect of various elements—Since only a single stage is involved in the reduction of tungsten and the factor for calculation is large, the method can be recommended only for the determination of large amounts of tungsten, *e.g.*, in tungsten concentrates and in ferro-tungsten. The possible effects of iron, molybdenum, chromium, and copper are important. *Iron*—The redox potentials of Fe^{II}/Fe^{III} (+ 0·75 v.) and W^{V}/W^{VI} (+ 0·26 v.) are so different that two clearly defined potential jumps are observed; thus, even with 10 ml. of 0·1 *N* iron alum solution the titration of 5 ml. of 0·1 *M* sodium tungstate in 100 ml. of concentrated hydrochloric acid is unaffected. If oxalic acid is also present, the reduction of iron and tungsten occurs simultaneously and slowly, and titration gives the combined iron and tungsten content. *Molybdenum*—In concentrated hydrochloric acid solution, molybdenum alone gives indications of the transitions $Mo^{VI} \rightarrow Mo^{V}$ and $Mo^{V} \rightarrow Mo^{III}$, but with tungsten also present no steps are found on the titration curves before the tungsten end-point, which then corresponds to reduction of tungsten to the quinquevalent stage and molybdenum to the tervalent stage. *Chromium*—The redox potential of $CrO_4''/Cr^{\cdots}$ is + 1·3 v.; chromates are therefore reduced well before tungsten and have no effect on the determination. *Copper* is first reduced to cuprous chloride and the conditions are then unfavourable for reduction to metal; no interference with the tungsten determination is observed.

Titration of reduced tungsten with an oxidising agent—For the determination of molybdenum and titanium in steels, etc., Klinger, Stengel, and Koch (*Arch. Eisenhüttenw.*, 1934–35, **10**, 433) reduced first with zinc and later with chromous chloride in excess; on addition of an oxidising agent two points were obtained on the titration curve, the first indicating oxidation of the excess of chromous chloride and the second, oxidation of the sought-for element. In a similar case, Sirokomski and Shukov (*Zavod. Lab.*, 1945, **11**, 373) used air to oxidise the excess of reducing agent. These methods are, however, unsuitable for tungsten, since in the former case the transition $Cr^{II} \rightarrow Cr^{III}$ is not detectable and in the latter, air oxidation may be used only when the redox potential is not less than + 0·4 v.; hence quinquevalent tungsten (+ 0·26 v.) is oxidised before the titrant is introduced. It is recommended, therefore, to reduce with chromous chloride under potentiometric control so that no excess is added, and to titrate the tungsten in the same apparatus by means of an oxidising agent (dichromate). For this, a standard solution of chromous chloride is not required; chromous chloride serves merely in place of solid reducing agents (zinc or amalgams), but its use, with a potentiometric end-point, ensures complete reduction of tungsten and avoids the need for separating the liquid. The oxidimetric titration is preferable to the reductometric since interference by other elements is less and fewer separations are required.

Procedure—Decomposition of the material (i) *Scheelite concentrate.* Heat 0·25 g. of the finely ground material in a small porcelain dish with 4 to 5 ml. of concentrated hydrochloric acid (addition of nitric acid is unnecessary), evaporate nearly to dryness, treat with 3 ml. of 20 per cent. sodium hydroxide solution, and, after solution of the tungstic acid, add 10 ml. of saturated oxalic acid solution to ensure a clear solution later, particularly in the presence of much iron. Transfer the solution to the titration vessel which already contains 100 ml. of concentrated hydrochloric acid. (ii) *Wolframite concentrate.* Dissolve 0·25 g. of the material, finely-ground in an agate mortar, in 25 ml. of concentrated hydrochloric acid in a 75- to 100-ml. beaker under cover. Heat gently and, after decomposition, add 3 ml. of concentrated nitric acid to attack pyrites. Evaporate nearly to dryness, and add formalin dropwise until the evolution of brown fumes ceases. Add to the nearly dry residue 3 ml. of 20 per cent. sodium hydroxide solution, mix to dissolve the tungstic acid, add 10 ml. of saturated oxalic acid solution, and pour into the titration vessel containing 100 ml. of concentrated hydrochloric acid. (iii) *Ferro-tungsten.* Treat 0·2 g. of the finely ground material with 2 or 3 ml. of hydrofluoric acid and a few drops of concentrated nitric acid in a platinum dish, and, after dissolution, add a few drops of formalin, and then 2 ml. of concentrated hydrochloric acid when the nitric acid has been fully decomposed. Evaporate nearly to dryness, add sodium hydroxide, and continue as described above.

Titration—(i) Direct titration. Connect up the titration cell, etc., as indicated above and titrate with 0·1 *N* chromous chloride solution (0·1 ml. is equivalent to 0·0184 g. of W or 0·0232 g. of WO_3) taking as initial point the potential jump due to iron. Standardise the chromous chloride solution each day on 0·1 *N* copper sulphate solution, using

10 to 15 ml. in 100 ml. of water containing 5 ml. of concentrated hydrochloric acid, heated to boiling during the titration. (ii) Dichromate titration of the reduced solution. Titrate potentiometrically with chromous chloride solution, which need not be standardised, until the potential jump due to tungsten is obtained. Replace the burette by one containing 0·1 *N* potassium dichromate and titrate to re-oxidise tungsten. G. S. S.

Determination of Gold by a Back-titration Method. L. I. Tzobar (*Zavod. Lab.*, 1946, **12**, 506–507)—A rapid, accurate, and economical method of determining gold in gold alloys is based on reduction with an excess of standard ferrous iron solution and titration of the residual ferrous iron with permanganate.

Procedure—Dissolve 0·1 g. of the sample in 4 to 5 ml. of *aqua regia* by heating on a sand-bath, evaporate to a small bulk, and, if the alloy contains no copper, add 2 to 3 ml. of 0·1 *N* cupric chloride, and then run in from a burette saturated sodium (or ammonium) bicarbonate solution until a precipitate forms and the yellowish liquid turns greenish. Clear the solution with 8 to 10 drops of 4 *N* sulphuric acid, boil and then cool, add 25 ml. of 0·1 *N* ferrous ammonium sulphate in dilute sulphuric acid, and shake for 30 sec. The precipitated gold quickly settles (the presence of silver chloride from any silver present in the alloy assists). Introduce 2 to 2·5 ml. of the Zimmermann-Reinhardt reagent, and titrate the excess of ferrous salt by means of 0·025 *N* potassium permanganate. G. S. S.

Silver Sulphide method for Determining Cadmium. N. N. Lapin (*Zavod. Lab.*, 1946, **12**, 158–160)—Although cadmium can be precipitated quantitatively by means of hydrogen sulphide, volumetric or other methods of determining cadmium based upon a determination of sulphide in the precipitate fail because the latter always contains compounds other than CdS, *e.g.*, Cd_2Cl_2S. Determination of cadmium in the precipitate by gravimetric means takes, however, considerable time. It is now shown that a rapid volumetric determination, accurate to 1 part in 100, can be carried out by treating the washed precipitate with a known volume of standard silver nitrate solution, filtering, and titrating the residual silver nitrate. The cadmium sulphide is transformed completely into insoluble silver sulphide and any chloride in the precipitate forms silver chloride, so that the amount of precipitated silver salts corresponds to that of the cadmium compounds in the precipitate decomposed.

Procedure—In 100 ml. of the solution (*e.g.*, of 0·3 g. of cadmium nitrate) mixed with 5 ml. of concentrated hydrochloric acid, precipitate cadmium sulphide by means of hydrogen sulphide, filter, and wash the precipitate until soluble chlorides and sulphides are removed. Transfer the precipitate and paper to a conical flask, add 30 ml. of 0·1 *N* silver nitrate, close the flask, shake vigorously for 3 to 5 min. to break up the paper and precipitate, remove and wash the stopper, heat the solution for 5 min. on an electric hot-plate, filter into a titration flask, washing all the soluble silver salts into the flask, and titrate with potassium thiocyanate solution, after adding 2 ml. of 10 per cent. iron alum solution and 2 ml. of concentrated nitric acid. Calculate the cadmium content from the amount of silver nitrate consumed. G. S. S.

Determination of Nickel and Cobalt in Iron Ores with Preliminary Removal of Iron by means of Sodium Fluoride. I. V. Tananaev and V. G. Silnitschenko (*Zavod. Lab.*, 1946, **12**, 140–141; *Metallurgia*, 1946, **35**, 122)—When iron has to be separated before a determination of nickel or cobalt, sodium fluoride may be used to give a dense, crystalline white precipitate of $5NaF.2FeF_3$ which is easily filtered off; it contains all the iron and negligible amounts of nickel and cobalt.

Procedure—With iron ores, boil 1 g. of the sample with 15 ml. of concentrated hydrochloric acid to complete decomposition, dilute to 100 ml. with water, and add aqueous ammonia until a precipitate appears. Clear the solution by adding a small amount of hydrochloric acid, and treat the hot solution with 100 to 120 ml. of a hot 4 per cent. solution of sodium fluoride. Filter after 8 to 10 min. In the filtrate determine (i) nickel by means of dimethylglyoxime, after adding aqueous ammonia or sodium acetate, or (ii) cobalt by making ammoniacal and electrolysing. G. S. S.

Separation of Niobium and Tantalum from Titanium. V. A. Oschman (*Zavod. Lab.*, 1946, **12**, 154–157)—Hydrolysis of niobium and tantalum salts in sulphuric acid solutions of low *p*H always gives incomplete precipitation of the earth acids, whilst at high *p*H, contamination by other components of the solution is apt to occur. In hydrochloric acid solutions of high concentration, however, these difficulties are largely removed, but large amounts of titanium and zirconium still interfere. It is now shown that, if the titanium is reduced to the tervalent state before hydrolysis, considerable quantities of titanium may be present without contaminating the earth acid precipitate obtained in hydrochloric acid solution, since tervalent titanium salts are more stable towards hydrolysis than are quadrivalent titanium salts (*cf.* ferrous and ferric salts). In the presence of large amounts of both titanium and zirconium, niobium is incompletely precipitated, but with zirconium alone, or in small amount accompanied by titanium, the interference is not serious.

Procedure—Treat 0·2 to 2 g. of sample (*e.g.*, ilmenite concentrate containing 10 per cent. of earth acids, $Nb_2O_5 + Ta_2O_5$, and up to 80 per cent. of titania, together with iron, etc.) with hydrofluoric acid and sulphuric acid to remove silica, fuse the dry residue with pyrosulphate, and extract with a mixture of 10 ml. of concentrated hydrochloric acid, 4 ml. of 30 per cent. hydrogen peroxide, and 36 ml. of water, warming gently but avoiding, as much as possible, decomposition of the peroxide. Cool, filter, and repeat the fusion on the insoluble matter if it is considered desirable. Add 5 ml. of hydrochloric acid (if a second fusion has been carried out, extract the melt with a mixture containing only 5 ml. of acid, and after mixing with the first extract, add no additional acid), transfer to a Someya reduction apparatus (or to a separating funnel, capacity 300 to 500 ml., joined below the tap to a test tube by means of a short rubber tube), containing 200 g. of pure zinc amalgam, remove air by means of carbon dioxide, close the reductor, and shake for 8 to 10 min. after the brown colour of ferric chloride has disappeared. During the shaking, the colour changes through violet (tervalent titanium) to green if niobium is present, the latter colour serving as an indication that reduction of titanium is complete; with tantalum minerals no green colour may appear, but 10 min. may be considered sufficient for the reduction of 1 g. of titanium dioxide if freshly prepared amalgam has been used (normally the amalgam may be used 5 to 6 times before replacement). Remove the amalgam from the reductor. If the separating funnel is used, the amalgam is allowed to flow into the test tube and is washed by the upward flow of water contained in the test tube, and the volume of the tube and rubber connection should be just sufficient to hold all the amalgam. Pour the solution into a beaker, if necessary through a filter, dilute with boiling water to 500 ml., heat quickly to the boiling point, and maintain the solution at this point for 20 min., then add some paper pulp, and filter through an 11-cm. paper containing paper pulp, washing with hot, diluted hydrochloric acid (4 : 100) until sulphates are removed. Ignite the paper and precipitate to constant weight, fuse the residue with 3 g. of pyrosulphate, extract the melt with 20 ml. of diluted sulphuric acid (1 : 3) containing 3 ml. of perhydrol, make up to 50 ml., and determine the titanium colorimetrically. To determine zirconium in the extract, add 10 ml. of 20 per cent. ammonium phosphate solution, maintain at 60° C. for 2 hr. (or, better, overnight), filter, wash the precipitate with 5 per cent. sulphuric acid containing perhydrol, and then with 4 per cent. ammonium nitrate solution, and ignite and weigh as ZrP_2O_7. Deduct from the weight of impure earth acids the weights of TiO_2 and ZrO_2 calculated from the results of the titanium and zirconium determinations.

Some typical results are—(i) Zirconium absent. $(NbTa)_2O_5$ taken 1·0 mg., found 1·0, 1·2 (TiO_2 500 mg.); taken 14·9 mg., found 15·3 (TiO_2 500 mg., Fe_2O_3 100 mg.); taken 197·9 mg., found 200·0 (TiO_2 1000 mg.). (ii) Moderate amounts of zirconium present. $(NbTa)_2O_5$ taken 1·0 mg., found 1·5 (TiO_2 200 mg., ZrO_2 10 mg.); taken 2·0 mg., found 2·1 (TiO_2 nil, ZrO_2 6 mg.); taken 4·9 mg., found 5·0 (TiO_2 0·1 mg., ZrO_2 15 mg.); taken 9·9 mg., found 9·4 (TiO_2 0·1 mg., ZrO_2 30 mg.). (iii) Titanium and zirconium present in large amounts. $(NbTa)_2O_5$ taken 7·9 mg., found 3·0 (TiO_2 400 mg., ZrO_2 50 mg.); taken 29·7 mg., found 13·6 (TiO_2 500 mg., ZrO_2 80 mg.); taken 59·4 mg., found 33·5 (TiO_2 500 mg., ZrO_3 150 mg.). G. S. S.

Agricultural

Rapid Semi-micro Volumetric Determination of Potash in Leaf Ash. G. W. Chapman (*J. Agric. Sci.*, 1947, **37**, 29–31)—Results with a maximum error of 1 per cent. may be obtained rapidly by the decomposition of potassium cobaltinitrite, precipitated by the usual methods, in the presence of disodium monohydrogen phosphate to prevent the formation of insoluble cobalt compounds during the formation of sodium nitrite. The cobalt phosphate formed is readily soluble in the dilute sulphuric acid used in the permanganate titration.

Procedure—Treat a sample containing 2 mg. of potassium oxide with 6 drops of a mixture of 1 part of sulphuric acid, 1 part of water, and 2 parts of hydrochloric acid, and digest for 1 hr. on the water-bath, and then for 1 hr. on an air-bath at 160° to 200° C. There should be no appreciable excess of sulphuric acid. Add 5 ml. of water, shake occasionally during 15 min., and pipette 2 ml. of the clear liquid into a 15-ml. centrifuge tube. Add 2 ml. of cobaltinitrite reagent (Lewis and Marmoy, *J. Soc. Chem. Ind.*, 1933, **52**, 177) quickly, and leave to stand for about 4 hr. to complete precipitation; decomposition occurs on standing for some days. Add a few drops of a 70 per cent. solution of camphor in ethanol to sink any precipitate that floats, and centrifuge for 5 min. in a centrifuge of 9-inch radius at 3000 r.p.m. Decant most of the liquid, add 5 ml. of 70 per cent. ethanol and centrifuge again. Drain off the liquid and wash twice more with ethanol, then drain thoroughly and dry in a water-oven.

Add 5 ml. of 30 per cent. disodium monohydrogen phosphate solution, stir, and digest on the water-bath for 15 min. to 1 hr. Transfer to a test tube, add an equal volume of diluted sulphuric acid (1 in 8), and titrate with standard permanganate. Use a 10-ml. burette graduated to 0·02 ml., and keep a layer of permanganate on the liquid surface until the approach of the end-point, which may be regarded as the formation of a colour lasting one minute.

The titration factor for the permanganate should be determined at the working temperature, a variation of 1·9 per cent. being obtained between results at 2° and 30° C. The cobaltinitrite reagent is stable at ordinary temperatures after an initial vigorous aeration for 3 hr. Loss of nitric oxide causes results obtained by titration in an open beaker to be 1·2 per cent. low. Calcium, magnesium, iron, and phosphorus, in normal amounts, do not interfere, but calcium gives a considerable positive correction if present in a several-hundred-fold excess. Ethanol, weaker than 70 per cent., gives less efficient centrifuging. M. E. D.

Water Analysis

Rapid Electrometric Determination of the Alkalinity of Sea Water. D. H. Anderson and R. J. Robinson (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 767–769)—The alkalinity of sea water (defined as the number of mg.-equivalents of hydrogen ion neutralised by one litre of sea water at 20° C.) is primarily due to bicarbonates, with lesser amounts of borates, phosphates, etc., forming a buffer system of *p*H about 8. Sea water is about as alkaline as 0·002 *N* sodium bicarbonate solution.

As measured with the glass electrode, $pH = -\log C_{H^{\cdot}} f_{H^{\cdot}}$, where $f_{H^{\cdot}}$ is an empirical constant determined as follows. Titrate 100-ml. samples of sea water of various ionic strengths with 0·01 *N* hydrochloric acid, using the glass electrode as indicator. Take the equivalence point (about *p*H 4·5) as occurring with the greatest change in e.m.f. per unit volume of added acid. Continue the titration to a measured *p*H of about 3 and calculate the normality $C_{H^{\cdot}}$ of the excess acid from $C_{H^{\cdot}}$ = (ml. of excess HCl) × (normality of HCl)/(ml. of sample + ml. of HCl). Then calculate $f_{H^{\cdot}}$ from the final *p*H.

The value of $f_{H^{\cdot}}$ depends upon the ionic strength of the sample and upon its *p*H at measurement. For a given ionic strength, however, $f_{H^{\cdot}}$ is sensibly constant in the approximate range *p*H 3 to 3·6. Examination of natural and artificial sea waters ranging in ionic strength from about 0·07 to 1·0 (corresponding to about 2 to 29 g. of chloride per litre) showed that $f_{H^{\cdot}}$ fell rapidly from 0·844 to about 0·76 (at a chloride concentration of approximately 7 g. per litre) and then remained sensibly constant. A graph or table relating its value to the chloride concentration may thus be prepared.

Procedure—Deliver 100 ml. of sea water into a glass-stoppered, 135-ml. bottle (previously aged for several months with hydrochloric acid of *p*H about 3·5) containing exactly 25 ml. of 0·01 *N* hydrochloric acid. The sample may then be retained for examination ashore. Bring to the desired temperature, measure the *p*H by means of the glass electrode and hence obtain $C_{H^{\cdot}} f_{H^{\cdot}}$. Then

$$\text{Alkalinity} = 2{\cdot}500 - {\cdot}1250\,(C_{H^{\cdot}} f_{H^{\cdot}})/f_{H^{\cdot}},$$

where $f_{H^{\cdot}}$ has the appropriate value for the sample. Alternatively, tables or graphs relating *p*H and alkalinity may be prepared, or the *p*H meter may be calibrated to read the alkalinity directly. The probable error is about 1 per cent.

The procedure should be applicable to effluents, biological fluids, etc., which are fairly uniform as to ionic strength and concentration of unknown material. J. T. S.

Method for Determining the Total Hardness of Water by means of Soap Solution. E. N. Teterkin (*Zavod. Lab.*, 1946, **12**, 177–178)—Total hardness is determined by comparing the amount of soap solution required to give a stable foam with the sample under test with that required with standards of known hardness. The method has certain advantages over the normal processes: any kind of commercial soap may be used; the standards are prepared from ordinary laboratory chemicals; the end-point is definite; and the difficulties due to the presence of magnesium in the sample and its absence in the standard are overcome by introducing magnesium ions into the standard.

Reagents and standards—Make up a soap solution about 1 to 2 per cent. (calculated after drying the soap for 24 hr. at 30° to 40° C.) in 40 per cent. alcohol, allow to stand for 1 to 2 days, and then filter through a thick filter paper. Prepare an artificial standard of hardness 100 degrees (German) containing magnesium corresponding to the quantity present (say, 25 per cent.) in the waters to be examined in the following way. Obtain a saturated solution of calcium oxide in water, filter, titrate 100 ml. of the filtrate with 0·1 *N* hydrochloric acid, calculate the result to German degrees of alkalinity (say A°), and place 75,000/A ml. in a graduated litre flask. Neutralise with the calculated quantity of 0·1 *N* hydrochloric acid, add 620 mg. of magnesium sulphate heptahydrate (dried at 140° C. to constant weight), and make up to the mark with distilled water. This solution has a hardness of 100 degrees. Construct calibration curves by taking different volumes of this solution corresponding to hardnesses from 1 to 15 degrees with 1 degree intervals, diluting each to 100 ml., and titrating at 18° to 20° C. with the soap solution to a stable foam which does not disappear from the surface during 5 min.; deduct the volume of soap solution required to give a foam with the distilled water used in the preparation of the standards. If the soap solution deposits flakes on keeping, clarify the solution at 30° to 50° C. before use.

Procedure—Titrate with the soap solution at 18° to 20° C., as for the standards and read off the hardness value from the calibration curve.

The method can be applied directly to water of hardness 15 German degrees and less; the error does not exceed 0·10 to 0·25 degree. It cannot be used for water containing much organic matter. G. S. S.

Review

TEXTBOOK OF PHARMACOGNOSY. By T. E. WALLIS. Pp. vi + 504. London: J. and A. Churchill. 1946. Price 28s.

"Pharmacognosy is the objective study of crude drugs of animal and vegetable origin treated scientifically." This sentence from the opening chapter of Dr. T. E. Wallis's new volume is the keynote of the book. His aim has been to produce a book on pharmacognosy giving a new presentation of the subject. The late Professor H. G. Greenish's "*Materia Medica*" was for many years one of the standard works and the publishers suggested that Dr. Wallis should use some of the figures and material from it. The result is that though this book may be regarded as the successor of Professor Greenish's volume, so well known to students, it is now brought up to date and in line with modern views on the subject.

In this work drugs exhibiting cellular structure are classified morphologically, while dried juices, extracts, etc., are grouped according to the method of their preparation. The simpler structures are dealt with in earlier chapters, and the author proceeds successively to more complex structures in the later portions of the book. Final chapters deal with Commerce and Cultivation, Transport and Marketing, the Acquisition of Diagnostic Characters, Deterioration and Storage, Insect and Other Pests, Adulteration and its Detection. An appendix deals with Calcium Oxalate Crystals. The book is well indexed and a glance through it shows the wide range of drugs covered.

Descriptions are systematised and the particulars arranged approximately according to their use in the identification and evaluation of the material in the group. Individual drugs are described under a number of headings in the following order: (1) Origin, (2) Cultivation and Preparation, (3) Characters, macroscopic and microscopic, (4) Constituents and Tests, (5) Evaluation, (6) Adulterants and Substitutes, and (7) Uses. The information given is comprehensive in its scope and fully up to date. The 213 illustrations and histological drawings are noteworthy, the latter (many the work of the author) being exceptionally clear.

Quantitative microscopy, for which the author is so well known and much of which he originated, is stressed where it is of diagnostic importance.

The work is well produced and of convenient size, and the use of different kinds of type makes it easy to pick out the information required under the appropriate heading. Both the author and the publishers are to be complimented on this volume, which will be of use not only to advanced students and workers in pharmacognosy, but also to analysts, pharmacists and others whose work is concerned with crude drugs. S. K. CREWS

SUPPLIES OF LABORATORY CHEMICALS

A FURTHER letter has been received from the Board of Trade to the effect that discussions have taken place with Chemical Suppliers and that a number of difficulties have been disposed of. It is believed that about half of the chemicals reported by members as being unobtainable are now available again. Difficulties presented by the other half are being tackled and it is hoped that the majority will be available in reasonable quantities in the not too distant future.

In order to be satisfied how effectively the position has been improved the Board has asked me to bring to their notice any information that comes before me showing if and where difficulty is still being found.

K. A. WILLIAMS,
Hon. Secretary

SEPTEMBER, 1947 Vol. 72, No. 858

THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS
AND OTHER ANALYTICAL CHEMISTS

Fluorimetric Analysis

The following four papers were read at a meeting of the Physical Methods Group in London, on February 11th, 1947.

Fluorimeter Design

By E. J. BOWEN

THE first consideration relating to fluorimeter design is whether the measurements should be by eye or by photo-cell. Visual measurements suffer from all the weaknesses of the human observer, while photo-electric methods can be carried out more accurately and rapidly, and with less fatigue. There are occasions, however, when visual methods may be preferred, if the solution contains unextracted impurities themselves fluorescing with a different colour. Changes of tint of the fluorescence then give warning that there is something unsatisfactory about the solutions. Instruments for visual work are the Pulfrich Photometer or adapted plunger-type colorimeters.

In photo-cell instruments the lamp, solution cell, and photo-cell may all be arranged in line, or the photo-cell may be arranged to collect the fluorescent light emerging at right angles to the exciting light beam. The latter arrangement seems preferable, as for very weak fluorescences it may not be possible to remove by filters all traces of the direct exciting light beam. In the right-angled arrangement up to four photo-cells could be used round the solution cell, though in practice two would probably be the optimum.

For very accurate work it is desirable to eliminate the effects of fluctuations in the intensity of the exciting-light source. This is most conveniently done by using a double apparatus to allow of balancing. Such an arrangement is illustrated in Fig. 1. Light from a central lamp L sends out two beams in opposite directions; these are made parallel by lenses and of appropriate wavelength by light-filters F_1. After passing diaphragms D_1 the beams traverse solution cells C. One cell contains a standard and the other the solution to be measured. These need not be solutions of the same substance if standards more stable than the solutions to be measured are better. The fluorescent light passes diaphragms D_2 and filters F_2 to reach photo-cells P. The photo-cell outputs are connected in opposition to a measuring device balanced to zero when identical solutions are placed in the cells C.

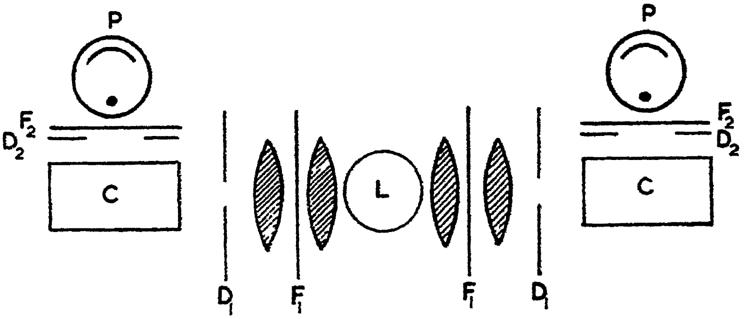


Fig 1 Fluorimeter viewed from above See Umberger and Lamer, *J. Amer. Chem. Soc.*, 1945, **67**, 11.

The following considerations relate to the various components.

Lamp—For most work the small high-pressure 125-watt mercury lamp is the best, since high-intensity monochromatic light, and long-wave ultra-violet light, are easily obtained from it. To avoid inconstancy of output the lamp should be provided with a stabilised voltage and guarded against irregular ventilation, since of the three lamp variables, voltage, current and temperature, only two are independent.

It may happen that the absorption band of a fluorescent solution, as of rubrene in hexane, lies in a region where the mercury lamp provides no line. A concentrated filament projection-type tungsten-filament lamp must then be used. This needs a copper sulphate solution filter to remove heat from the light beam and some care in design to dissipate the great heat in the lamp housing.

Filters F_1—For measurements at low concentrations the filters F_1 should transmit a spectral region more or less coincident with a high or a maximum value of the absorption band of the solution. Using the mercury lamp it is roughly true that colourless solutions (even when concentrated) need the 3650 A. line, and yellow solutions the 4358 A. line. The presence of nitrites, sulphites, and other light-absorbing substances in the solution may render the use of the 3650 A. line impossible even though it may fit best the absorption band of the fluorescent substance. In some circumstances the mercury line 4047 A. might be very useful, but it is not easy to isolate at high intensity.

Gelatin-film (between glass) filters are commonly used because they can be obtained in a greater range than coloured glass filters. When exposed to the full radiation of the mercury lamp, however, they may show rapid deterioration. It is very desirable to interpose an ultra-violet absorbing glass filter such as Chance OY9 or 10 to minimise this trouble.

Solution cells—Given sufficiently sensitive detecting instruments, the ultimate sensitivity of fluorimetry is set by five factors:

(*a*) Rayleigh and Raman scattering from the liquid.
(*b*) Scattering from dust particles.
(*c*) Scattered light from reflections from the cell walls.
(*d*) Fluorescence of the solvent.
(*e*) Fluorescence of the cell walls.

Present-day sensitivities are so far from the limit set by (*a*) that this may be left out of consideration. Factors (*b*) and (*d*) can be minimised by careful purification and technique. Factor (*e*) is a matter of careful choice of material. Glass fluoresces owing to the presence of traces of certain elements such as manganese, cerium, samarium or europium, and the production of "non-fluorescent" glass needs the co-operation of the glass manufacturers. Scattered light from the cell walls is probably the largest source of error in most instruments. The following devices would reduce this trouble.

(*a*) The use of a cell shaped like a cross as shown in Fig. 2, constructed of opaque material with four clear plane faces.
(*b*) Scrupulous care in avoiding surface contamination and scratches in the conventional type of rectangular cell.
(*c*) Careful adjustment of lenses and of diaphragms D_1 to limit the path of the light-beam to the centre part of cell C.
(*d*) The use of very thick glass ends to the cell C on the exciting light axis, or, alternatively, immersion of C in a larger rectangular trough containing a liquid, so that the light-scattering planes where the exciting light enters and leaves the solution are kept well away from being "viewed" by the photo-cell at P.

Balancing of photo-cells—There are various ways of balancing the photo-cells and of obtaining a measure of the fluorescence. The reading may be taken either directly from the opposed photo-cell outputs when unknown and standard solutions are placed one in each of the cells C, or the detecting instrument in the photo-cell circuit may be brought to zero by adjusting

(*a*) a variable calibrated diaphragm in a D_1 position, or
(*b*) a variable and graduated resistance or potentiometer in the electrical circuit.

The relative merits of these methods have never been fully assessed.

Filters F_2—These may be gelatin-film filters chosen to transmit the fluorescent light while removing the exciting wavelengths. Were these 100 per cent. efficient, errors due [illegible] scattered exciting light would disappear. There is usually need to off-set loss of light by using not very dense filters.

Photo-cells—The barrier-layer type of photo-cells has the advantage of not needing an external source of electrical supply. They are not sensitive enough, however, for work with feebly fluorescent solutions. Vacuum-type alkali-metal cells, chosen for the wavelength range of the fluorescence to be studied (*i.e.*, potassium for blue and green, rubidium for orange, and caesium for red) are then necessary Their outputs are amplified, either by the D.C. method with an electrometer valve (preferably enclosed with photo-cell and high resistance in an evacuated glass envelope) or by A C. methods with light-choppers interposed in the light beam. A new development, however, is now on the way. Nine-stage photo-electron multipliers, such as the American R.C.A. tube 931 A, provide exceedingly high sensitivities to light over the whole spectral range, and are capable of measuring to 10^{-7} foot-candles or less. Used with a spectrometer they permit the direct measurement of Raman lines from liquids which need quite long exposures of a photographic plate. As it is very desirable to cut down the intensity of the exciting light beam to avoid photochemical changes in unstable solutions, these new detectors will provide greater sensitivity with lessened risk of destruction of fluorescent material. One may even forecast a fluorimeter of the future with a spectrometer interposed between the solution and the multiplier cell, whereby both the intensity and the wavelength distribution of a feeble fluorescence may be simultaneously determined.

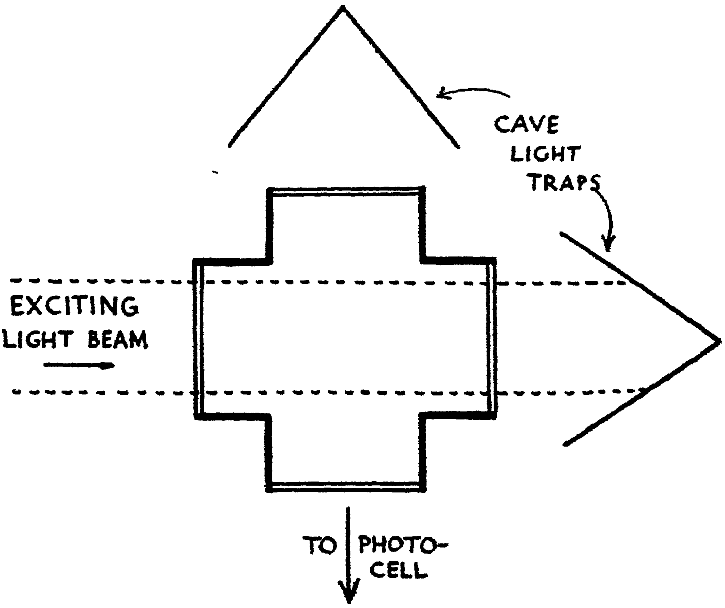


Fig. 2.

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Fluorescence Quenching in Solutions

By E. J. BOWEN

The mechanism of fluorescence is given the following interpretation on present-day theory.

When a molecule absorbs a quantum of light it receives a large accession of energy, and "jumps" in the very short time interval of 10^{-15} seconds to an *electronically excited state*, one of its electrons having assumed a new wave-pattern or *orbital*. If undisturbed, this excited state persists for a time interval of about 10^{-8} seconds on the average and then re-radiates all or almost all of the absorbed light energy. In a liquid, molecules collide in time intervals of about 10^{-12} seconds Excited molecules of most substances lose all their energy as *heat* before they have lived long enough to radiate, either because of energy transformations caused by collisions or because of internal energy changes. Such substances do not show the phenomenon of fluorescence. Only molecules having peculiarly stable excited states can resist de-activating effects for long. These are usually either ions such as rare-earths or $UO_2^{\cdot\cdot}$, where the excited electron is one from an inner shell relatively protected from outside influences, or "conjugated" organic substances with systems of "non-localised" electrons where the excitation is "spread" and "thinned out" over a large number of linkages.

The intensity of fluorescence is strictly proportional to the amount of light absorbed. "Saturation" could be produced only at light intensities many thousand times greater than at present can be used. For analytical work dilute solutions are employed, where the fraction of light absorbed, and hence the fluorescence, is sensibly directly proportional to the concentration of fluorescent substance, or should be. When two solutions with concentrations in the ratio of 2 : 1 are compared, however, often the fluorescent intensity ratios are less than 2 : 1. For very dilute solutions this error may arise from fluorescence of the glass cells, unwanted scattering, etc. In stronger solutions there may be a real deviation from the 2 : 1 ratio of intensities, owing to the effects described below. The term "quenching" has been used to cover all these effects; it seems better however to restrict its meaning to cases where a real non-proportionality of fluorescence to concentration in dilute solutions exists. In an ideal case of unquenched fluorescence, each absorbed light quantum should lead to the emission of a quantum of fluorescent light, and the "fluorescence efficiency", (emitted quanta)/(absorbed quanta), should be unity. Although the absolute values of fluorescence efficiencies are not easy to measure, it puts matters on a sounder basis to define "quenching" as any effect reducing a fluorescence efficiency below unity. On this view all non-fluorescent coloured substances are 100 per cent. quenched. With this definition the molecular picture of the quenching process can be classified into a number of types:

1. Intramolecular effects.
2. Intermolecular effects:
    - (*a*) Bimolecular collisions,
        - (i) with solvent molecules,
        - (ii) with solute molecules, *i.e.*, with added "quenchers" or with other molecules of the fluorescent substance.
    - (*b*) Compound-formation in solution,
        - (i) with solvent molecules,
        - (ii) with solute molecules, *i.e.*, with added substances or with other molecules of the fluorescent substance.
3. Inner filter action of solvent or added substances.

The term "quenching" is narrowed by some workers to effects under 2; sometimes to 2 (*a*) only.

Inner filter action, 3, is merely the diminution of fluorescence of a substance in solution through the presence of a "coloured" solvent or other solute whereby part either of the absorbed or of the fluorescent light is wasted, almost as if an absorbing screen were interposed in the light beam. It occurs only when the absorption by the "coloured" substance is not small, and could be recognised by detectable changes in the light absorption.

Compound-formation in solution, 2 (*b*), refers to chemical equilibria established in the solution before the fluorescence is examined, and must carefully be distinguished from photochemical action, which causes fluorescence to diminish with *time* but is not a true *quenching* effect. Many fluorescent dyes in aqueous solution, *e.g.*, uranin, are less fluorescent in concentrated solutions. This is associated with a change of absorption spectrum due to *dimer* (or *polymer*) formation by a thermal equilibrium. The dimer molecules are not fluorescent and act as inner filters. The effect is much more marked in aqueous than in alcoholic solutions, *i.e.*, in the liquid of higher dielectric constant, and therefore the forces holding the dimer together cannot be "dipole" forces; wave-mechanical "dispersion" forces or "hydrogen bonding" are probably the cause. In some cases, *e.g.*, the quenching of the fluorescence of anthracene in benzene solution by added bromobenzene, the "compound-formation" is not apparent from very noticeable changes in the absorption spectrum, but the effect can be shown to be 2 (*b*) (ii) and not 2 (*a*) (ii) because the quenching effect *diminishes with rise of temperature.* This is due to the break-up of the "compounds" at higher temperatures so that a greater fraction of the absorbed light is available for free anthracene molecules. By applying the Van t'Hoff isochore the heat of formation of the compound is obtainable from the temperature coefficient of quenching, and values of 0·5 to 2·0 k. cal./mole are obtained for different systems.

Experimentally, collisional quenching, 2 (*a*), is distinguishable from 2 (*b*) because it *increases* with rise of temperature. Both 2 (*a*) (ii) and 2 (*b*) (ii) obey the equation $(F_0 - F)/F = k\,[Q]$, where F_0 and F are the fluorescence intensities without and with added quencher, and [Q] is the quencher concentration; k is called the "quenching constant." In *gaseous* systems k is the product of collisional frequency and probability of quenching on one collision, but in *liquids* the *collisional* must be replaced by the *encounter* frequency. In all measurable examples of collisional quenching by added substances in solution the probability of quenching on one collision is high. Collisions in liquids differ from those in gases in that neighbouring molecules make many "repeated" collisions before separating; such a group of collisions is called an "encounter." If quenching occurs early in the course of an encounter the quenching constant will be determined by the *encounter* and not the *collisional* rate, and the former is a function of the *viscosity* of the liquid. It has been shown that

$$k = 7 \times 10^{21} PR^3 \left(\frac{Dt}{R^2} + \frac{\sqrt{Dt}}{R}\right)$$

approximately, where P = probability of quenching during one encounter, R = sum of radii of fluorescent and quenching molecules, = about 7×10^{-8} cm., t = half-life of fluorescent molecule = 10^{-8} sec. approximately, and D = diffusional constant of solute molecules, proportional to (absolute temperature)/(viscosity). The second term of the equation becomes important only in viscous liquids. This "collisional" type of quenching is shown by dissolved oxygen in solutions of fluorescent aromatic hydrocarbons; the quenching is strong in fluid hexane solutions and small in viscous paraffin solutions. For ordinary values of the molecular constants the maximum value of k is about 150, *i.e.*, such quenching becomes inappreciable at a quencher concentration below 10^{-2} g./litre.

Quenching processes 2 (*a*) and 2 (*b*) appear to be markedly distinct from each other; (*a*) depends on encounter rate and therefore on viscosity, (*b*) depends on mass-action constants of compound-formation and on inner filter action, and they have opposite temperature effects. In spite of this one cannot attribute fundamentally important differences to the two classes. For solutions of anthracene, carbon tetrachloride, bromoform and acetylene tetrabromide are "collisional" quenchers, while bromobenzene, *iso*propyl bromide and trimethylene dibromide are "compound" quenchers. The difference seems to arise solely from the life-time of the "compound" and its relation to the half-life of the fluorescent molecule. Nitrobenzene behaves as a "compound" quencher for solutions of rubrene; *m*-dinitro- and *s*-trinitrobenzenes, though usually forming more stable compounds with aromatic hydrocarbons, here act as "collisional" quenchers, evidently because of stereochemical interference effects of the phenyl groups of the rubrene with the nitro groups of the two latter substances.

Intramolecular effects grouped under 1 are difficult to distinguish from 2 (*a*) (i). Here the excitation energy is supposed to be degraded to heat by some internal rearrangement of energy within the molecule. The temperature coefficient might be expected to be small, but no clear experimental test has yet been applied to recognise this class. In both classes the polarity or dielectric constant of the solvent might in some instances be an important factor through its influence on the "transition probability" of the passage of the molecule from its excited potential energy surface to the ground one. A polar solvent might (compared to a non-polar one) increase or decrease quenching effects (*a*) by raising or lowering the "crossing points" of such surfaces, or (*b*) by changing the "transition strength" between the upper and lower states. Here again little quantitative application is available for fluorescent solutions.

Sharply to be distinguished from the quenching effects discussed above are *photochemical reactions* occurring between molecules in solution, where the changes increase with *time*. Such reactions may or may not be observed as subsequent effects to intermolecular quenching processes of group 2, and should not *themselves* be referred to as "quenching." A great many of the photochemical effects observed in solutions of fluorescent substances are *electron-transfer* reactions, *e.g.*, in solutions of thionine to which ferrous ions have been added. Fluorescence quenching occurs, accompanied by a photochemical reaction whereby the thionine molecules (T) excited by light receive an electron from a ferrous ion, changing to $T' + Fe^{\cdot\cdot\cdot}$. The leuco-form of the dye is then produced by the reactions:

$$T' + H^{\cdot} \rightleftharpoons TH \text{ (semi-quinone)}$$

and

$$2TH \rightarrow TH_2 + T$$

Electron-accepting ions or molecules may also quench with photo-reaction to give positive dye ions. In different examples of fluorescent solutions reversible or irreversible destruction of the fluorescent substance may be observed, and photosensitisation often occurs, *i.e.*, a permanent change in some added substance in the solution is brought about while the concentration of fluorescent material remains unchanged. These effects can all be explained by variations in the secondary changes following on the initial electron-transfer reaction associated with the fluorescence quenching. The term "fatigue" has sometimes been used to cover examples of fluorescence weakening with exposure to light and recovering in the dark. Occasionally it has been applied to irreversible effects, but it seems anyhow a poor term to employ when ordinary photochemical nomenclature is available, and is better left to describe effects in solid phosphors, where the actual processes taking place are much more obscure.

Dissolved oxygen quenches the fluorescence of some solutions, particularly of aromatic hydrocarbons. Continued exposure may lead to irreversible oxidation effects. If easily oxidisable substances are simultaneously present, photosensitised oxidation of the latter may occur, the fluorescent substance remaining unchanged. In these circumstances an *increase* of fluorescence may be observed with time of exposure, owing to the consumption of the oxygen and the consequent elimination of its effect as a collisional quencher.

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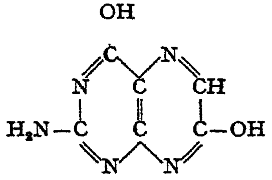
Applications of Fluorimetric Analysis to the Study of Pterins

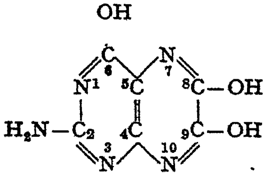
BY (MISS) D. M. SIMPSON

DURING the last few years interest in the study of the pterins has increased enormously as it has become apparent that this group of substances is widely distributed in living matter. Interest has been further stimulated by the recognition that pterins may be used to treat certain anaemias in animals and that the molecule of folic acid contains xanthopterin.[1]

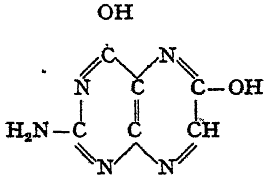
The study of the pterins began with the work of Hopkins,[2] who in 1895 isolated materials, later shown to be leucopterin and xanthopterin, from the wings of butterflies. During the years 1925 to 1944, workers in the schools of Wieland, Purrmann, Schöpf and Koschara succeeded in isolating a number of pterins from natural sources, in determining their structural formulae and in achieving their synthesis. (For a summary of this work, see [3].) . The most important and widely distributed pterins are leucopterin, xanthopterin (9-deoxyleucopterin, uropterin) and anhydroleucopterin (8-deoxyleucopterin, *iso*xanthopterin).



Anhydroleucopterin



Leucopterin



Xanthopterin

Various derivatives of these substances have been prepared, such as carboxylic acids and thiopterins with sulphur at the 2-carbon atom.[4]

One of the most characteristic properties of the pterins is that, unlike the purines, they show a pronounced fluorescence in solution, even at high dilutions; some pterins also fluoresce in the solid state. This suggests that the fluorescence spectra could conveniently be used as a means of identifying pterins in mixtures; further the intensity of fluorescence might, with suitable precautions, be employed to determine the concentration of pterin present.

The earlier workers, for example Schöpf and Becker,[5] contented themselves with describing the colour of the fluorescence as observed visually. This procedure has its limitations as the apparent tint is considerably affected by the colour of the solution. Hüttel and Sprengling[6] determined roughly the region over which fluorescence was observed for ichthyopterin, a material isolated from the skin of members of Cyprinidae, with which the fluorescyanine of Polonovski and his co-workers is probably identical.[7]

It is clear that if fluorescence spectra are to be used as a means of identifying pterins, the first requirement is that these spectra should be accurately determined. Since the fluorescence spectrum of a pterin differs according to whether it is solid or in solution, and varies with the *p*H of the solution, standard spectra must be obtained for a suitable range of conditions. It is also desirable to use light of a definite wavelength, for example Hg 3650 A, to excite the fluorescence. Standard spectra are at present available only for leucopterin and xanthopterin.[3]

The meagre data on pure pterins do not allow any generalisation to be made on the question of the relationship between fluorescence spectrum and chemical constitution. It may, however, be pointed out that the fluorescence spectra of leucopterin and xanthopterin in solution are of the same family type, both showing two strong bands between 4358 and 5461 A. These bands do not appear to be markedly changed even when there is evidence that the molecules are present in a combined state, though they show slight variations, presumably owing to salt formation, if the *p*H of the solution is altered. The ichthyopterin of Hüttel and Sprengling,[6] a derivative of anhydroleucopterin, shows in solution at *p*H 4 to 8, bands at 4500 to 4700 A. (strong, maximum 4316 to 4323 A.[7]) and 6900 A. (weak). A pterin from the eyes of the minnow (*Phoxinus phoxinus*), kindly provided by Mrs. Pirie (Nuffield Institute of Ophthalmology, Oxford), shows a different type of spectrum, with bands at 4200 to 4600 A. (strong, maximum 4400 A.) and 5250 A. (weak) in neutral solution. There is thus no reason to expect that the fluorescence spectra of all pterins should be similar. Polonovski and his co-workers[4] suggest that fluorescence is associated with the grouping $=N-\underset{|}{C}-N=$ in the first ring. If this structure is absent, as in some of the thiopterins, the fluorescence disappears.

It is probably unwise to identify pterins solely by means of their fluorescence spectra. These spectra consist of broad bands; it is difficult to determine the positions of the maxima with very great precision, and the absolute intensities are not known. On the other hand there is comparatively little interference from other substances. The ultra-violet absorption spectra of pterins, as of most complicated molecules, also consist of broad bands, but they may be obtained quantitatively and with much greater accuracy; most substances, however, show some absorption in the region 2000 to 7000 A. The identification of a pterin in a mixture should be made, not only by means of the fluorescence spectrum, but also by using the ultra-violet absorption spectrum and the chemical properties of the material; if all three lines of evidence are in agreement then the identification may be considered reliable.

Most biological materials show some fluorescence under ultra-violet light. This is frequently confined to the blue parts of the spectrum well away from the strong characteristic pterin bands, so that little interference is experienced. Thus it was possible on the basis of the chemical evidence and the ultra-violet and fluorescence spectra to conclude that the argentaffine cells of the stomach and intestine contain xanthopterin, probably as a complex.[3] In this connection it should be noted that the dry preparations show fluorescence, although solid synthetic xanthopterin does not fluoresce.

In the investigation of the fluorescent material in liver extracts other difficulties are encountered.[8] The riboflavine present produces an intense band in the same region 5200 to 5800 A (maximum 5300 A) as that of one of the xanthopterin and leucopterin bands. Happily the shape of this band is very characteristic, and it can be effectively suppressed by working in normal acid solution. Examination of a large number of liver extracts showed that the position and relative intensity of those bands of their fluorescence spectra not due to riboflavine were remarkably constant. This suggested that the same fluorescent material was responsible in every instance. These bands correspond most closely to those of leucopterin, though they appear over a *p*H range greater than that of its solubility. This makes it probable that the leucopterin, if present, is in the form of a complex. In this case it would not, however, be justifiable to identify the fluorescent material definitely as leucopterin, until it has been obtained in a much higher state of purity, so that confirmatory evidence may be derived from its chemical properties and ultra-violet absorption spectrum.

On the other hand the fluorescent material isolated from the eye of the dog fish (*Squalus acanthias*) was successfully shown to consist mainly of xanthopterin, on the basis of its fluorescence and ultra-violet spectra and its chemical behaviour.[9]

An interesting problem in this connection is the constitution of the so-called uric acid pigment.* This curious red material, which is readily soluble in water and in acid and alkaline media, is obtained by heating uric acid with water in sealed tubes at 200° C. for some hours. Hopkins[2,10], from an examination of its chemical properties suggested that it contains a pterin, probably xanthopterin. The ultra-violet absorption spectrum of a specimen kindly provided by Dr. Quibell (Manchester University) is very similar to that of xanthopterin. The fluorescence spectra in solution show bands at:

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* The work on the spectra of this material, which has not been previously published, was carried out in collaboration with Dr. W. Jacobson (Strangeways Research Laboratory, Cambridge).

4650 A. (mS),	5300 A. (S),	5680 A. (mS),	5900 A. (mS),	6200 A. (W);	$N/2$ NaOH;
4560 A. (S),	5170 A. (mS);				neutral;
4590 A. (S),	5200 A. (mS),		5920 A. (W);		20% H_2SO_4.

(S) = strong; (MS) = moderately strong; (W) = weak

The material certainly contained uric acid as an impurity, which would not, however, have appreciably affected its fluorescence spectra, as the latter substance shows a very faint blue fluorescence in alkaline media only. The positions of the bands of the uric acid pigment in neutral and acid solution correspond to those of xanthopterin, in agreement with the conclusions of Hopkins. The relative intensity of the bands is different; this is to be expected as the solutions are red, indicating absorption in the blue and yellow which would lead to self-absorption of the fluorescent light. This inner filter effect has also been observed with impure dark coloured specimens of xanthopterin. The fluorescence spectrum of the uric acid pigment in alkaline solution does not correspond to that of xanthopterin. It shows a remarkable increase in intensity in the region 5100 to 6000 A., indicating that some other material fluorescent at *p*H > 7 must be present. It is impossible to suggest what this may be, though it can be stated with some certainty that it is not leucopterin.

The quantitative analysis of solutions containing pterins by means of fluorescence spectra is a much more difficult problem. Use of fluorescence for quantitative purposes implies that the exciting light must not be so strongly absorbed that the fluorescence is confined to a thin layer at the side of the cell nearest to the exciting beam. This difficulty is encountered particularly in dealing with dark coloured solutions such as some commercial liver extracts.[8] The dilution required to ensure that there is no appreciable drop in fluorescence intensity across the cell is so large that the resultant errors in calculating concentrations may be considerable. High dilutions are also required to eliminate self-quenching and the absorption of fluorescent light by the solution itself. This last effect was very pronounced in the solutions of impure xanthopterin and uric acid pigment. A rough calculation for leucopterin, based on its observed molecular extinction coefficient ($\epsilon \sim 10^3$ at 3600 A.[6]) and molecular weight (195), suggests that if Hg 3650 A. is used as exciting radiation the concentration to be employed is of the order of milligrams per litre, if the fluorescent light is observed over the full width of a 1-cm. cell.

Not many attempts have been made to determine pterin concentrations by means of fluorescence spectra. The work of Koschara, von der Seipen, and Aldred,[11] who found the combined concentration of xanthopterin and riboflavine in urine from the intensity of fluorescence, is probably unreliable, as one would not expect the total intensity to be necessarily proportional to the total concentration if more than one fluorescent material were present.

The work of Decker,[12] who used the total intensity of fluorescence to measure the concentration of leucopterin in various natural materials, is typical of the case in which the true chemical nature of the fluorescent substance is known. He determined calibration curves for intensity of fluorescence plotted against leucopterin concentration for synthetic material in solutions of known *p*H, and compared the fluorescence of his unknown preparations with that of the standard solutions.

This method could be improved if, instead of the total intensity of fluorescence, the intensity at a particular wavelength (preferably that of the maximum of one of the characteristic bands) were used to compare standard and unknown solutions. This would seem to be the only really reliable method to employ.

It is sometimes possible to use fluorescence spectra to determine relative concentrations even if the exact chemical nature of the fluorescent material is not known, when it is of course impossible to obtain standard spectra for comparison. Such a procedure is only justifiable if it is clear that interfering fluorescence is absent. In the case of the liver extracts previously discussed, the intensity of fluorescence observed, when that due to the riboflavine is suitably suppressed, runs parallel to the haemopoetic activity of these preparations, which strongly suggests that both fluorescence and activity are due to one and the same substance. The intensity of fluorescence of these materials may thus be used as a measure of their clinical activity.[8] This is a very valuable conclusion, as the intensity of fluorescence may be rapidly found, whereas previously only complicated clinical and biological methods have been available for the assessment of clinical activity. The determination of the absolute concentration of active substance in such preparations cannot be carried out until the exact chemical nature and molecular weight of the fluorescent material have been established, and until pure specimens are available to provide comparison spectra.

SUMMARY

Fluorescence spectra alone are insufficient to identify pterins in complex mixtures; ultra-violet absorption spectra and chemical tests should also be employed. Standard fluorescence spectra of synthetic pterins are at present available only for xanthopterin and leucopterin.

Using the complementory methods listed above, it has been possible to demonstrate the presence of pterins in such diverse materials as the argentaffine cells of the stomach and intestine, the fluorescent substance obtained from the eye of the dog fish (*Squalus acanthias*) and the so-called uric acid pigment.

To date, few quantitative determinations of the concentrations of pterins have been made by means of fluorescence spectra. Provided that suitable precautions are employed to avoid quenching and inner filter effects, this should be feasible if synthetic material is available for comparison. Examination of a large number of liver extracts suggests that the intensity of fluorescence might be used as a measure of the clinical activity of these preparations.

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Some Applications of Fluorimetry in Vitamin Analysis

BY E. KODICEK

IT is well known that a number of vitamins or their derivatives fluoresce. This fluorescence has been used in several instances for their quantitative estimation. Table I gives a list of vitamins or related compounds that show a fluorescence or develop it on appropriate treatment.

TABLE I

FLUORESCENCE OF VITAMINS AND RELATED COMPOUNDS

	Fluorescent compounds	Fluorescence (under specified conditions)
Vitamin B_1	Thiochrome	Blue
Riboflavine..	Riboflavine	Yellowish-green
Pyridoxal	Pyridoxal	Blue
Nicotinamide	CNBr complex	Blue
N-Methylnicotinamide	F_2	Blue
Co-zymase	F_c	Blue
Folic acid	Folic acid	Blue
Vitamin A	Vitamin A	Yellow

I propose to deal principally with vitamin B_1, riboflavine, N-methylnicotinamide ("N-MN") and nicotinamide—with short references to N-methylnicotinuric acid betaine and co-zymase, which we have also studied at Cambridge. For the estimation of these we have worked out methods which in our experience over several years have proved satisfactory for a considerable variety of materials. Without going into details of procedure, I propose to concentrate on the principles involved and to point out the advantages and disadvantages of the methods we use.

The requirements that should be fulfilled in an ideal method are as follows.

(1) RELIABILITY AND ACCURACY—

(*a*) The results should be repeatable with small error, say, ± 5 per cent. standard deviation.

(*b*) Recovery of added vitamin should be complete.

(*c*) The method should be specific, and should agree with at least one other method, preferably biological. Treatment of extracts should ensure that only the vitamin is present in solution or that other fluorescent substances that may interfere are as far as possible removed. These interfering substances may be either quite unrelated to the vitamin, or structurally related but biologically inactive. The blank, treated as far as possible in the same way as the unknown, should correct the error due to any unspecific fluorescence remaining in the extract.

(*d*) The extract should contain all the vitamin in its fluorescent form.

(*e*) The extract should be clear, colourless and free from the so-called "quenching" effect.

(*f*) Internal standards should be used in order to eliminate changes of fluorescence for reasons outside our control. By this I mean the standard solution should be estimated in presence of the unknown.

(2) EASE AND RAPIDITY—

(*a*) As few steps as possible should be employed and the simplest treatment adopted

(*b*) Techniques that may involve losses outside our control should be omitted. For example, in the use of adsorption procedures, variations in the properties of the adsorbing material or in the nature of the extract may result in ineffective adsorption by, or incomplete elution from, the adsorbent.

Having indicated the requirements of an ideal fluorimetric method, we will now consider our procedures.

VITAMIN B_1

We use the method of Harris and Wang,[1,2,3,4,5] the well-known principle of which consist. in conversion of vitamin B_1 to thiochrome (Jansen[6]) by oxidation with alkaline ferricyanides The steps are as follows.

(1) Extraction of the material at acid *p*H on the water-bath, and digestion with takadiastase and papain to break up the bound vitamin B_1 which may be present as co-carboxylase.

(2) Preliminary washing with *iso*butanol to remove interfering substances.

(3) Rapid oxidation by alkaline ferricyanide in presence of methanol, followed by treatment with 30 per cent. hydrogen peroxide.

(4) Extraction of the formed thiochrome with *iso*butanol and clarification of the *iso*butanol layer with absolute ethanol.

(5) Use of an extract treated similarly but without oxidation, as the "blank."

(6) Finally, matching of the *iso*butanol layer for its fluorescence, either visually or photometrically.

The standard deviation for determinations on numerous samples by this method has been found to be of the order of ± 5 per cent. Vitamin B_1 can be recovered completely. The vitamin-B_1 potency of numerous samples of foodstuffs estimated by this method has agreed well with that found by the biological bradycardia method, and also by growth and convulsions.[2] The preliminary washing with *iso*butanol was found to be essential; it removes most of the interfering material. Rapid oxidation by adding sodium hydroxide followed by ferricyanide is necessary to obtain reliable results. The presence of methanol stabilises the conversion of vitamin B_1 to thiochrome. With regard to the blank, the use of hydrogen peroxide eliminates a great part of the undesired fluorescence and renders matching much easier. Nevertheless, there are one or two materials that give a fluorescence of a different tint from that of the thiochrome standard, rendering the results unreliable. For such materials it is best to use a visual method, as the difficulties are at once obvious and can nearly always be overcome by suitable procedures, *e.g.*, washing with light petroleum or excess of hydrogen peroxide. A photo-cell, on the other hand, will record the total fluorescence, limited of course by the filters used, but the result will not accurately represent the vitamin B_1 content of the extract.

It has been reported that N-methylnicotinamide can seriously interfere with the assay of vitamin B_1. However, the interference is not very serious. It will be seen from Table II that 4 μg. of N-MN per ml., a concentration likely to occur in normal urine, does not alter the fluorimetric readings of vitamin B_1 found by the method of Harris and Wang.

TABLE II

FLUORIMETER READINGS OF VITAMIN B_1 SOLUTIONS WITH AND WITHOUT N-METHYLNICOTINAMIDE (N-MN)

(Filters Wratten 49A + 2A)

	Treatment	Fluorimeter readings mm.
1 ml. of water	Blank	1
" + 4 μg. of N-MN	Blank	1·5
" "	Oxidised	1·5
" + 0·4 μg. of vitamin B_1	Blank	2
" "	Oxidised	13
" " + 4 μg. of N-MN	Blank	1
" " "	Oxidised	12

With regard to the extraction, no hard and fast rule can be laid down as to how to extract efficiently every possible material. The best procedure has to be worked out for each separately.

The final extracts obtained by this method are clear. Internal standards are always used.

The method is comparatively simple and quick. It does not rely on adsorption procedures. There are one or two materials, for example blood, for which the method has so far not proved satisfactory. Blood seems to contain substances that either destroy the vitamin B_1 during the preparation of extracts or else quench the fluorescence. Good recoveries could not be obtained.

RIBOFLAVINE

The method we have worked out (Kodicek and Wang[7]) uses the yellow-green fluorescence of riboflavine excited by blue light. The principle of the procedure is as follows.

(1) Extraction on the water-bath with 0·1 *N* hydrochloric acid. Precipitation of proteins by metaphosphoric acid and/or digestion with takadiastase when starchy materials are assayed.

(2) Washing of the extract with chloroform at acid *p*H and oxidation with saturated potassium permanganate solution followed by treatment with hydrogen peroxide to decolorise the remaining permanganate.

(3) The extracts are adjusted to *p*H 6; methanol is added to clarify them if necessary.

(4) Fluori-photometric readings are taken, with blue filters for the incident light and yellow filters between the solution and photo-cell. A sensitive photo-cell galvanometer circuit is used.

(5) In the blank, the riboflavine is reduced to a non-fluorescent compound with a neutralised solution of sodium hyposulphite ($Na_2S_2O_4$).

For a great number of materials the results are reproducible with a standard error of about ± 5 per cent. With foods of low riboflavine content, for instance white flour, the error is greater but does not exceed 10 per cent. Added riboflavine can be recovered completely. The method is satisfactorily specific. The results agree well with those of microbiological assays. Washing with chloroform removes most of the interfering fluorescence. The oxidation with permanganate and hydrogen peroxide decolorises the extract almost completely. Nevertheless, a certain amount of residual unspecific fluorescence remains in some extracts. Allowance is made for it by the blank procedures.

It is important to point out that when hyposulphite ($Na_2S_2O_4$) is used as the reducing agent in the blank, blue light is essential for obtaining correct readings. We have observed that under ultra-violet light sodium hyposulphite diminishes very strongly, or extinguishes almost completely, the total fluorescence of the extract. When a cell containing this reducing agent is placed in a beam of ultra-violet light in front of a cell containing riboflavine, for instance, the solution does not fluoresce. But when blue light is used as the exciting source the fluorescence is almost unaltered.

The extraction of riboflavine, when combined with enzymic digestion, seems to be complete. The resulting extract is clear and almost colourless. Quenching is observed in many instances and is allowed for by use of an internal standard. The method is quick and easily carried out. Adsorption procedures were found to be unnecessary when a sensitive galvanometer circuit was used. We employ a direct-reading fluorimeter of the Cohen type, giving a deflection of about 100 mm. for 0·05 μg. of riboflavine per ml. of final extract.

N-METHYLNICOTINAMIDE (N-MN)

We have modified (see Kodicek and Carpenter[8]) the ketone procedure recommended for urine by Huff, Perlzweig, and Tilden,[9] for the estimation of N-MN, which has been shown to be an important metabolite of nicotinic acid. The principle of the method is as follows. N-Methylnicotinamide in aqueous alkaline solution reacts with ketones to produce a greenish-blue fluorescence. On acidification the fluorescence changes to blue, and is intensified by heating. We use methyl ethyl ketone instead of acetone because it gives greater fluorimeter readings and is less volatile, and have modified the procedure for the blank (see below).

The specificity of the method was tried by testing a number of compounds, of which only co-zymase and N-methyl-nicotinuric acid betaine were converted into fluorescent compounds. They can be eliminated by deducting the residual fluorescence of a filtrate passed through a Decalso column, as, unlike N-MN, they are not adsorbed. For urine, with which we could find little or no fluorescence in Decalso filtrates, another simpler blank has been adopted; the addition of sodium hydroxide is omitted and the reaction conducted throughout at an acid *p*H at which N-MN does not develop a fluorescence. Internal standards are used for reference. Recovery of added N-MN is complete. The advantages of the method are that it is quick and easily performed. The one-phase procedure reduces the possible errors.

We have tested extracts obtained from liver and found that by far the greater portion of the fluorescence remains in the Decalso filtrates and therefore cannot be due to N-MN. The fluorescent compound in these extracts behaves like the samples of co-zymase we have tested. Measurement of this fluorescence might form the basis of a method for estimating co-enzymes.

NICOTINAMIDE

Scudi[10] has observed that nicotinamide when treated with cyanogen bromide, and then extracted at an alkaline *p*H with *iso*butanol, fluoresces like F_2, the compound formed from N-methylnicotinamide. We have applied the ketone method mentioned above to the estimation of nicotinamide. Pure nicotinamide can be estimated readily when the reaction is allowed to proceed at an alkaline *p*H and the fluorescence is measured at this *p*H.

Procedure—Take 10 ml. of $M/15$ phosphate solution (*p*H 6·8) containing 50 to 100 μg. of nicotinamide and add 4 ml. of freshly prepared cyanogen bromide solution (see Wang and Kodicek[11]). Heat for 4 minutes in a water-bath at 56° C. Cool for 5 minutes and make up to 15 ml. with water.

Take 5 ml. of this solution, add 2 ml. of methyl ethyl ketone and 1 ml. of 5 N sodium hydroxide and make up to 30 ml. with water. Keep the solution in the dark for 3·5 hours. Then read on a photo-electric fluorimeter with Wood's filter for the incident light and Wratten No. 47 and Calorex filters between solution and photo-cell.

The fluorescence under these conditions reaches its maximum after 3·5 hours and declines slowly within the next 2 hours. The intensity of the fluorescence is proportional to the concentration of nicotinamide (Fig. 1).

Blanks and specificity—Two types of blanks were investigated: (1) omitting cyanogen bromide—"No CNBr" blank, and (2) omitting 5 N sodium hydroxide—"No NaOH" blank.

Nicotinamide does not fluoresce under the conditions of either of the blank procedures. To test the specificity of the method and of both blanks, a number of miscellaneous compounds were investigated. The following compounds, at a concentration of 1 μg. per ml. of final solution, gave no fluorescence: nicotinic acid, coramine, trigonelline, α-picolinic acid, aminopyridine, nipecotic acid, pyridine, nicotine, sulphapyridine, nucleic acid, adenylic acid, indole-3-acetic acid, 1-methyl-indole-3-acetic acid, glucose, urea, uric acid, choline, inositol, pantothenic acid and *p*-aminobenzoic acid.

Other compounds tested showed a fluorescence (Table III). The most intense fluorescence was produced by vitamin B_1, but it could be differentiated from that due to nicotinamide by its development in the "No NaOH" blank. Folic acid and pyridoxal gave a fluorescence which increased when sodium hydroxide was omitted ("No NaOH" blank).

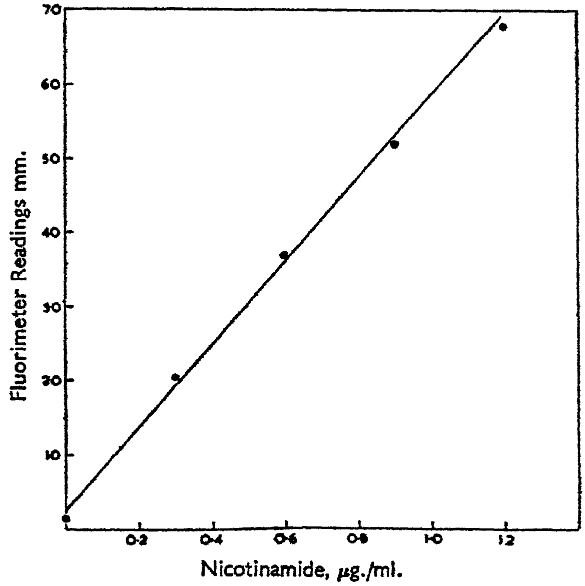

Fig. 1. Standard curve of fluorescence of nicotinamide.

N-Methylnicotinamide chloride and co-zymase showed a slight fluorescence which—unlike that of nicotinamide—persisted even when the cyanogen bromide treatment had been omitted ("No CNBr" blank).

TABLE III

Material 1 µg./ml. of final solution	Fluorimeter readings, mm.		
	Full procedure	"No CNBr" blank	"No NaOH" blank
Nicotinamide	62	2	2
N-Methylnicotinamide	10	10	2·5
Co-zymase (48 per cent. purity)	5·5	5·5	2
Quinolinic acid	6·5	1·5	1·5
Vitamin B_1	109	15	109
Pyridoxal	8	3·5	19
Folic acid	6·5	4·5	9·5

The procedure has not yet been fully developed for the estimation of nicotinamide in biological material. For analytical purposes where a differentiation between nicotinic acid and its amide is needed, the method may be found useful.

SUMMARY

A critical survey of some fluorimetric methods for the estimation of vitamin B_1, riboflavine, N-methylnicotinamide and nicotinamide has been presented.

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DUNN NUTRITIONAL LABORATORY
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DISCUSSION ON THE PRECEDING PAPERS

The CHAIRMAN, Mr. R. C. CHIRNSIDE, said that the Group had been very fortunate in persuading experts to come and deal with this subject. Mr. Bowen had dealt with fluorescence in general, and Dr. Kodicek and Dr. Simpson had described two special applications. The subject was of considerable interest at the moment, especially, he understood, for those people who had to test for vitamins under the new Orders of the Ministry of Food, and no doubt there were members present who were interested in other applications of the subject. The four papers would be discussed together.

Mr. WINCH said that he felt rather an intruder at the gathering and had not expected to be called upon to open the discussion. He was very interested, however, in the possibility of applying his knowledge of photo-electric photometric techniques to problems of fluorimetric measurement. He had developed for photometric, spectro-photometric and colorimetric purposes a photometer unit consisting of an emissive-type photo-electric cell together with an electrometer triode and associated grid leak resistance, all mounted in one evacuated tubular bulb. This was operated in a valve bridge D.C. amplifier circuit, the controls of which were in a separate cabinet connected to a mains-operated power unit and precision voltage stabiliser. The whole equipment, described as a Universal Photo-electric Photometer, had now been commercialised, and in this form had a limiting sensitivity of 10^{-8} lumens. Under laboratory conditions considerably higher sensitivity had been achieved. This instrument had proved a useful tool in a large number of applications to special photometric problems, and he thought that it might prove very helpful in fluorimetric investigations.

Mr. Bowen's proposed optical system for a photo-electric fluorimeter appeared in general to be sound, provided stray light was prevented from reaching the photo-electric cells. Mr. Winch saw no real difficulty in providing effective screening that would achieve this latter requirement. Mr. B. S. Cooper, a colleague of his, had recently collaborated with him in some interesting experimental fluorescence measurements utilising a Universal Photo-electric Photometer, and he suggested that Mr. Cooper might like to give details of what had been done.

Mr. B. S. COOPER said that although his experience was confined to one day's work in practical fluorimetry, he had felt that with Mr. Winch's expert photometric knowledge something might be done to increase the sensitivity and accuracy of fluorimetric estimation. A preliminary trial had been carried out at Kings Langley in collaboration with Dr. Wokes, and it was clear that the Universal Photo-electric Photometer had sufficient sensitivity to cover the requirements. A limit, however, is imposed by certain inadequacies in the design of most commercial fluorimeters and by certain unexplained fluorescence at the lowest concentrations. Later, some tests were carried out at Wembley to determine the useful range of sensitivity obtainable in an experimental fluorimeter incorporating a Universal Photo-electric Photometer. The apparatus, as arranged, used a 125-watt high-pressure mercury lamp, a Corning filter (No. 5860, 5 mm. thick) to isolate the 365-mμ. radiation and a large liquid cell, 10-cm. cube. The fluorescent radiation emerged through a Chance OY12 filter (a non-fluorescent chromium glass, opaque to near U.V. and violet radiation) and was received by the most sensitive photometer unit (100,000 megohms) that Mr. Winch could provide. The geometry of the apparatus and the photometric screening was such that the photo-cell could receive only the fluorescent light from the liquid. Using quinine sulphate supplied by Dr. Wokes, it was easy to obtain a reading (2·0) with a concentration as low as 1 millimicrogram per ml., but it was important to note that distilled water alone gave a reading almost as high, *viz.*, 1·7. At these very low concentrations therefore they were trying to measure something that gave effects very little different from those of distilled water.

Another matter worth noting was that the Chance filters OY9 and OY10 which had been mentioned were both fluorescent, probably owing to the presence of cadmium sulphide. The exit filter in a fluorimeter system should be non-fluorescent, such as the Chance OY12 already mentioned, although this filter was rather dense for the purpose. From a recent informal conversation he had the impression that Chance Bros. would be prepared to make filters suitable for fluorimetry if enough people were interested.

In connection with double-beam instruments, although in certain circumstances they were the most satisfactory, it should not be forgotten that the double-beam principle did not correct for every possible fluctuation of the light source. With a discharge lamp, particularly of the high pressure type such as was used to get high beam intensity, the fluctuation might be a movement of the luminous column rather than a change in its intensity, in which case the double-beam principle would not give perfect compensation.

Dr. F. WOKES admitted his responsibility for introducing to THE ANALYST the term "quenching" as applied to various deviations from linearity in fluorescence calibrations. He asked Mr. Bowen to suggest an alternative title to describe such deviations when they did not comply with the latter's precise definition of "quenching." Mr. Bowen had referred to the temperature effect. Some figures for the magnitude of this under different working conditions would be of great value to those who were trying to improve the accuracy of fluorimetric assays. The various instrumental errors described by Mr. Bowen could be best explored by calibration curves. It was regrettable that in many publications on fluorimetric analysis calibration data were lacking.

Dr. Kodicek had referred to the use of an internal standard in different fluorimetric assays. Could this be more precisely described. The blank used by Dr. Kodicek did not appear to allow for interfering substances produced by the oxidation of ferricyanide. In his second slide the reading of 1 mm. for the water blank seemed rather high in comparison with the reading of 13 mm. given by the 0·4 μg. of aneurine. This would suggest a possible error of 7 or 8 per cent. and also a low sensitivity for the fluorimeter.

Dr. Simpson should be congratulated on the excellent series of fluorescence spectra shown. Had she observed in her absorption spectra the usual mirror image relationship with the corresponding fluorescence spectra?

Mr. Bowen, in reply to Mr. Cooper, said that he would like to suggest that the residual factor might still be the effect from the cell or it might be the Raman radiation from the water. Presumably with a very dense filter all radiation scatter would be cut out; the Raman radiation would pass the filter.

Mr. Cooper said that although he was not certain, he thought that the residual fluorescence was continuous radiation and not a line spectrum. This was based on visual observation through a series of narrow band colour filters. He agreed, however, that it might possibly be Raman radiation.

Mr. Bowen said that some years ago some French investigators worked on very feeble effects from alkalis. They shone a light into a cell and made a very long exposure at right angles and got what they called fluorescence, but what amount, if any, was Raman radiation he did not know. Raman radiation was polarised. Except in highly viscous media, the molecule turned round in several rotations before it emitted fluorescence, which was therefore unpolarised. If nicol prisms were inserted it was possible to distinguish between Raman radiation and fluorescence.

He fully agreed with what had been said about instrumental error. No design was free from zero error. That was a common feature. The error might be due to impurities or to the glass, or a number of things might cause the curve to deviate from linearity. He could not conceive any better term than "instrumental error."

As for temperature effect, one got a big temperature effect only with inter-molecular quenching. In a solution like that of fluorescein, for example, there was not a very big temperature effect, but with a quenched solution, if it was of the bimolecular collision type, the temperature effect was quite high, though generally smaller if it was of the bond type—of the order of 10 per cent. for 20° C. Therefore there could be considerable errors, but they occurred only in solutions. Concerning the glass filters, he suggested that OY9 and 10 filters could be used in the main light beam.

Mr. Cooper said that a fluorescent filter might be objectionable even in the incident beam, since the fluorescent radiation could get into the liquid cell. In any event Chance OY9 and OY10 could not be used in the incident beam if near ultra-violet radiation were required.

Dr. Kodicek said that Dr. Wokes had asked him about an internal standard. By this he had meant the addition of standards to the unknown. He had not had time to go into details. The method they were using for thiochrome was the Harris—Wang method, which meant a visual method; therefore, in his Table II, which represented the experiments done visually, he had wanted to show actual numbers and figures. The absorbent filters Wratten 49 were used, and thus the sensitivity was low. It was distinct enough to obtain what he wanted in order to show the difference. It was in the range that he had mentioned.

Dr. Simpson, in reply to the question asked of her by Dr. Wokes, said that she did not think the absorption curves had been investigated at sufficiently low optical densities. The pterins had strong bands in the ultra-violet, and the spectra had not been observed at wavelengths as great as 4000 A.

Mr. H. N. Ridyard emphasised the importance of Mr. Bowen's remarks on the use of the term "quenching." This is used in very many papers in a loose manner to cover losses in fluorescence due to a variety of quite different causes, whereas photo-chemists have always used it in the restricted sense defined by Mr. Bowen. As a result, different effects have been confused and attempts to overcome or allow for these frustrated in consequence. For the same reason he would object to the alternative general term "instrumental error," suggested by Mr. Bowen, since this suggests difficulties lying in the apparatus. He would prefer some term such as "fluorescence inefficiency" which left to more precise terms the description of where the losses occurred.

This had a connection also with the method of using internal standards advocated by Dr. Kodicek and widely used: this is not always as strictly accurate as it is sometimes assumed to be, and the rigorous use of external and internal standards would help to an appreciation of the sources of error.

Mr. Cooper had called attention to the wandering beam of a discharge lamp in relation to the use of photo-cells placed on opposite sides of the light sources. He (the speaker) had always disliked this arrangement, and some years ago he employed in photochemical work a thin glass or quartz plate placed at 45° to the light beam to obtain a measure of the beam actually used. He had been interested to note that this arrangement has been employed in at least one recently described fluorimeter.

Dr. White said that he was in the position of being faced with a number of fluorimetric problems in the near future, and he had been particularly interested in two that Mr. Bowen had mentioned, one concerning quenching, and the other, particularly, his analysis. He thought that his method of looking at the various phenomena might be of some value, particularly in laboratories where the advantage of spectrographic methods was available, in distinguishing the fluoroscopic portions of different materials. He would like to suggest that Mr. Bowen should pursue this matter perhaps a little more deeply in the form of a communication of some sort to one of the journals, in which the ideas could be put forward for other people to develop on a practical basis. He thought that Mr. Bowen had got something there of real value to some of those concerned in fluorimetric work.

Dr. J. G. A. Griffiths asked if there were any substances that exhibited a fluorescence efficiency of unity. Where the fluorescence efficiency was less than unity was there not a probability that there would be a serious decrease in concentration of the substance under examination, as a result of photochemical reaction, in the course of the fluorescence measurements?

Mr. Bowen, in reply, spoke of the difficulty of measuring fluorescence efficiency. The geometry was awkward, and, secondly, it was necessary to compare very feeble quantities of light of different wavelengths. It had been done in a number of instances with the brilliant fluorescent dyes. He thought the fluorescence efficiency of rhodamine was 0·4 and those of certain of the yellow dyes were of the order of 0·8.

With regard to the photo-chemical changes that occurred, it was necessary to be clear in one's mind about the different possibilities of fluorescence quenching. There were some dyes—alizarin for example—completely non-fluorescent under any conditions, and that presumably was an intramolecular effect occurring in 10^{-15} sec. It was practically inconceivable that this activated molecule could react with dissolved oxygen. The collisions between molecules and solvents were about 10^{-12} sec., but the fluorescent molecule had a period of 10^{-8} sec., a relatively long period compared with collisions of a solvent molecule. There were thus a large number of possibilities of quenching. There could be quenching with and without photo-chemical action. The change could be reversible or non-reversible. All this could be explained in terms of suitable chemical equations which had been put forward by a number of people.

Dr. A. H. Gillieson mentioned the use of the American R.C.A. I.P.21, 22 or 28 electron multiplier tubes with electronically stabilised high-tension source, with which linearity over a range of 100,000-fold could be achieved. That type of instrument had been used for measurements of inorganic fluorescences in solid solutions down to an absolute amount of 10^{-12} g. of fluorescent substance, and was relatively easy to construct. Three laboratories were developing this type of instrument in this country—at the moment for fluorescence in solid solutions but fluorescence in liquid solutions would be undertaken.

Dr. P. Ellinger said he would like to make the following comments on Mr. Bowen's first paper. (1) He did not agree that photo-cells should be arranged at right angles to the incident beam. If the primary source of light was not extremely powerful and a considerable proportion of the primary beam was absorbed by the solution to be tested, the secondary beam might shift to the proximal side and reach only one end of the photo-cell, the conditions being uncontrollable. With an arrangement of the photo-cell in line with the primary, and sets of satisfactory filters as those made by Corning or Kodak, rays of the primary beam reaching the photo-cell were of very low, constant and measurable intensity, and the values could be deducted from those from the secondary light source. (2) With highly sensitive selenium rectifier cells, sufficient sensitivity could be obtained to measure very minute amounts of fluorescent material, *e.g.*, thiochrome in *iso*butanol down to 0·26 µg. can be measured with an accuracy of about ± 5 per cent. The advantage of the rectifier cell is that it can be brought much nearer to the source of the secondary light than the vacuum cell. An increase of sensitivity of the photo-cell or the galvanometer was undesirable because it increased simultaneously the instrument errors which were the greatest source of difficulties and inaccuracies. (3) As to whether photo-electric or visual fluorimetry was preferable one should keep in mind that the human eye was at least as sensitive as the best photo-cell, particularly in the green and yellow. With good sets of standards very good results could be obtained with visual measurement. (4) The question how the currents of the two photo-cells should be balanced for measurement had not been mentioned. The most reliable way was by electrical balancing which did not interfere with the optical arrangement; unfortunately this was not the usual method of balancing in the instruments sold commercially. Most photo-cells were not evenly sensitive over the whole area and altered their resistance when the size of the exposed area was altered. Methods of balancing that interfere with the aperture of the beam should, therefore, be avoided. (5) The question should be discussed under which circumstances fluorimetry or ordinary absorptiometry should be used. Fluorimetry was preferable only if absorption was in a spectral region where the sensitivity of eye or photo-cell was nil or very low and the emission spectrum was in a region of high sensitivity of the analysers. In all other circumstances ordinary absorptiometry was preferable.

Mr. Bowen, in answer to the last point, said that he had always imagined, perhaps wrongly, that absorption photometry was used for rather higher concentrations and fluorimetry below those concentrations. Fluorimetry could be used right down to the limits of instrumental error. In his earlier remarks he was envisaging that work was always done in fluorimetry with such dilute solutions as he had been speaking of; he did not think it should ever be used for cases of fluorescence confined to the cell wall.

Dr. S. Y. Thompson said that in attempts to increase the sensitivity of fluorimeters used by him for riboflavine work he had found the limiting factor to be the fluorescence of practically all the glass or gelatin colour filters available in this country, particularly when the blue mercury line 4358A was used.

Mr. Cooper said that by using filters in the manner he had described fluorescence of the filters themselves was eliminated. The last filter in the optical path was not fluorescing and also had the proper characteristics for absorbing unwanted radiation, hence all that was measured was the fluorescence of the liquid itself.

Mr. E. C. Slater said that for the past five years he had used an American instrument similar in design to that described by Mr. Bowen. Its sensitivity, expressed as the concentration that would give a galvanometer deflection of 1 mm., was 0·002 µg. per ml. for quinine sulphate in 0·1 *N* sulphuric acid (Corning 597 lamp filter, Corning 038 + Wratton 47 photo-cell filters) and 0·0002 g. per ml. for fluorescein (Corning 554 lamp filter, Corning 038 + 351 photo-cell filters). This sensitivity was found sufficient for vitamin work. He had found that with this instrument the readings were quite critically dependent on the volume of the solution in the cell (capacity 25 ml.), being maximum with 20 ml. Although there was an obvious explanation of the rise to the maximum he could not see why there should be a subsequent fall. Could Mr. Bowen suggest an explanation?

Mr. Bowen suggested that it was an effect due to reflection either of the incident light or of some of the fluorescent light, depending on the level of the liquid surface.

Dr. S. Judd Lewis suggested that it should be very helpful to refer fluorimetric comparisons to standard substances as controls on the optical methods using the photo-electric cell. For example, mannitol always exhibits the same fluorimetric values, whether it is purified by three or four recrystallisations from alcohol or water; the latter medium is perhaps the safer (see *J. Soc. Chem. Ind.*, 1944, 158). References to such a standard substance may be more reliable than those made by physical measurements. The two systems should be studied in relation to each other.

A Rapid Qualitative Test for Added Iron in Flour

By W. J. S. PRINGLE

Qualitative tests for the presence of added nicotinic acid[1] and vitamin B_1[2] have been published and a test for added iron is now described. It has been found sufficiently sensitive to detect also accidental contamination of flour with iron from the rolls and other machinery in the mill.[3]

The test depends upon the formation of a mauve iron lake with alizarin (1 : 2-dihydroxyanthraquinone). In analytical chemistry alizarin is mainly used as a reagent for aluminium, with which it forms a red lake, but this element is not likely to be a source of interference in the present test as it is added to flour only to a limited extent, in America as a self-raising agent, in the form of sodium aluminium sulphate. Manganese also forms a lake with the reagent, but the natural manganese, iron and aluminium contents of the flour either do not react with the alizarin under the conditions of the test or only react to a limited degree, so that the effect of these natural flour constituents is negligible.

PROCEDURE—

Soak acid-washed filter papers in a filtered saturated alcoholic solution of alizarin, remove them from the solution and allow them to drain for a few moments; then place them on glass plates and dry them over a hot plate or in an oven. After cooling, moisten the dried paper (which should now have a uniform brownish-yellow colour) on the glass plate with just enough *N* hydrochloric acid to wet it thoroughly. For a 9-cm. Whatman No. 30 paper 1·25 ml. is sufficient. Place 2 or 3 g. of the flour to be tested on a small sieve (1½ in. diameter) covered with silk bolting cloth, and distribute it evenly over the filter paper by tapping the sieve while moving it about over the paper. When sufficient flour has been added to absorb all the acid (this is shown by the flour covering the surface of the paper remaining dry) blow off the excess flour and wipe the glass plate clean up to the edge of the paper. After waiting 5 to 10 minutes to allow the iron to be dissolved by the acid, invert the glass plate and hold it over the mouth of an open bottle of concentrated ammonia solution. Move the plate about to allow the ammonia vapour to come in contact with all parts of the filter paper. After a few moments the adhering side of the filter paper as seen through the glass plate begins to turn a violet colour owing to the formation of ammonium alizarinate. When all the acid has been neutralised the filter paper has a uniform violet colour all over and is ready for drying. The drying may be carried out over a hot plate or in an oven (preferably with a glass door). It is important that the heating should be stopped as soon as the paper is dry, as over-heating causes the formation of a brown coloration which obscures the test. The heating should be stopped when the filter paper starts to buckle and leave the surface of the glass plate.

The violet colour of the ammonium alizarinate is discharged on drying, leaving the coloured iron compound unchanged against the original brownish-yellow background. The form which the coloured areas take is dependent on the nature of the source of the iron that has been added to the flour. If the iron has been added in powder form (either as reduced iron or sodium iron pyrophosphate) the coloured areas will appear as spots with intensely coloured centre and a paler coloured surrounding circle. The size of the spot depends upon the length of time allowed to elapse between the addition of the flour to the acid-soaked paper and the neutralisation of the acid with the ammonia vapour. Each spot indicates the locus of an individual particle of added iron or iron salt. If, on the other hand, an iron solution has been sprayed on to the flour, coloured spots will still be formed, but they will be considerably larger in area and of approximately even intensity throughout.

THIOCYANATE TEST—

A simple test for iron-enriched flour that has recently been described[4] depends upon the use of 5 per cent. potassium thiocyanate solution in N hydrochloric acid. In this method the enriched flour and a control (unenriched) flour are slicked side by side in the manner used for the Pekar test[5] and the reagent is added with a dropper at the junction of the two flours, in amounts sufficient to wet an area approximately 1 in. in diameter. Allow to stand for at least 10 minutes. "In the event that added iron compounds are present, a deeper red colour will be found than in the case of the unenriched flour. Small local areas of intense red show up after 20 minutes, indicating the location of individual particles of iron."

The need to wait 20 minutes for the full result of the test is due to the fact that usually the iron is added in the ferrous condition and is only slowly changed to the ferric state after being brought into solution by the acid component of the reagent. Thiocyanate reacts only with ferric iron, and we have found that the test may be speeded up and made much more sensitive if an oxidising agent is added to the acid thiocyanate reagent. If equal quantities of a 2 per cent. solution of ammonium persulphate and the acid thiocyanate reagent are mixed and this new reagent is used for the test the full result is apparent after two minutes. An additional improvement to the test is the substitution of the technique of the Pekar test for wetting the surface of the flour, instead of the addition of the reagent from a dropper. The slicked flours are carefully slid into the new reagent solution and then removed and the excess of reagent is wiped away. This ensures more even wetting of the flours and prevents the break-up of the smooth surface which often occurs when a dropper is used.

The thiocyanate test may also be carried out in the manner described above for the alizarin test. A dry filter paper is wetted with 1·25 ml. of the new reagent and the flour to be tested is sprinkled on by means of the sieve. This method has the advantage that smaller quantities of sample and of reagent are required.

SUMMARY—

Two methods have been described for the detection of added iron or its salts in flour.

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THE RESEARCH ASSOCIATION OF BRITISH FLOUR MILLERS
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ST. ALBANS, HERTS.

March, 1947

Note

A SIMPLE DIRECT-READING SEMI-MICRO BALANCE

IN a recent investigation involving numerous preparations on a semi-micro scale, weighings were considerably facilitated by the construction of the simple direct-reading balance shown in Fig. 1. This has a capacity of about 0 to 80 mg., but for other purposes the sensitivity may be increased or decreased.

The essential parts of the apparatus are of glass. A self-locating fulcrum system centres the pointer with respect to the scale. No arrestment is thus necessary; when the weighing spoon is removed, the counterpoise end of the beam comes to rest upon the glass-covered surface of the wooden upright. To prevent interference by draughts the balance is enclosed in a small wooden box, the front of which is glazed and opens outwards in the manner of a door.

The beam, weighing spoon support, pointer and damping device are constructed from a single piece of 3-mm. diameter glass rod, as shown in Fig. 2. The pointer is formed by drawing down the rod to about 0·8 mm. diameter and bending as shown. To construct the fulcrum or "knife edge" a piece of 3 mm. rod is drawn out at both ends and bent twice at right angles, as shown at a. The extremities of the fulcrum are then fused until hemispherical, care being taken that the "legs" are of the same length. Sealing wax is used to cement the finished fulcrum to the beam.

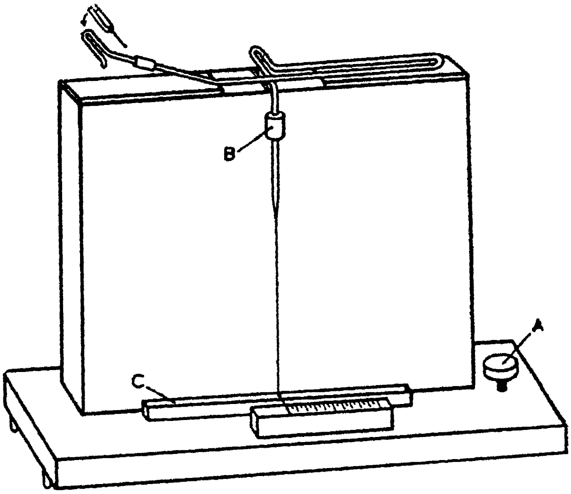

Fig. 1.

A small glass trough cemented in a groove cut across the top of the wooden upright serves as a bearing surface for the fulcrum. Stages in construction of this trough are shown in Fig. 3 A hole about 3 to 4 mm. in diameter is first blown in the wall of a length of 8-mm. outside-diameter glass tubing, as shown at a. The tubing is then heated in the region of the hole and the ends are pulled apart, as shown at b. When cold, about 3 cm. of the narrow central portion, which has a cross-section shown at c, is cut out, and, by heating carefully in the blowpipe flame, the ends are closed to form a small "boat" about 25 mm. long and about 2·5 mm. wide. The finished component is depicted at d.

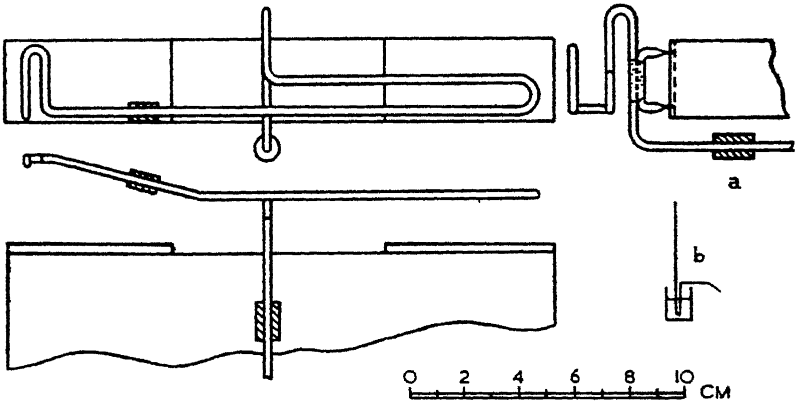

Fig. 2.

Weighing is carried out on small glass spoons, which are very convenient for picking up the material to be weighed and for transferring it to the reaction vessel when the weighing is complete. Details of construction are shown in Fig. 4. U-section glass, prepared in a similar manner to that described above, but of larger size, is employed. If soft glass is not objected to, a batswing burner is preferable to a blowpipe in the fabrication of these larger sections. Handles consisting of 2-cm. lengths of 1·5 mm. diameter glass rod are attached to the rear ends of the spoons. By cutting small pieces from the ends of the handles, the spoons are adjusted to be equal in weight; when the adjustment is complete, the ends of the handles are rounded in the blowpipe flame.

A paper scale graduated in 2-mg. divisions and covered with sheet celluloid is mounted on the wooden base (Fig. 1) so that the pointer moves close to its surface but does not touch. To enable the zero to be adjusted, a tilting device A is fitted to the base. This consists of a short length of 2-B.A. brass screw rod which may be rotated by means of the knob. The rod passes through the base and then through a nut soldered to a piece of sheet brass secured to the underside of the base. The end of the rod then makes contact with the bottom of the balance case. Rotation of the knob tilts the base as required; the method of adjustment is thus similar to levelling up an ordinary balance equipped with a plummet.

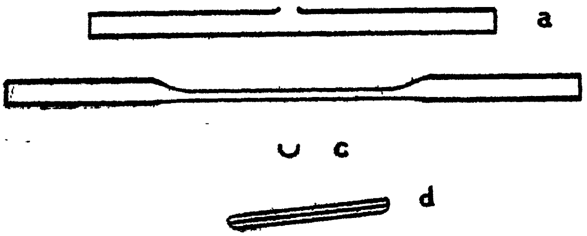

Fig. 3.

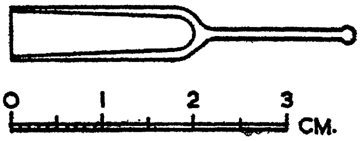

Fig. 4.

Counterweight B consists of a 2-cm. length of rubber pressure tubing which is split longitudinally. It is sprung into position on the upper part of the pointer and may be slid up or down, thus allowing the sensitivity of the balance to be adjusted.

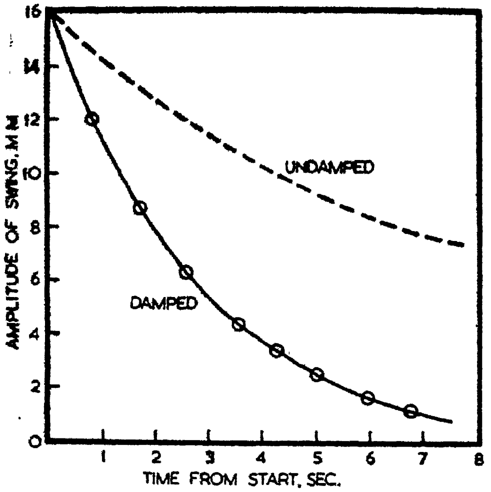

Fig. 5.

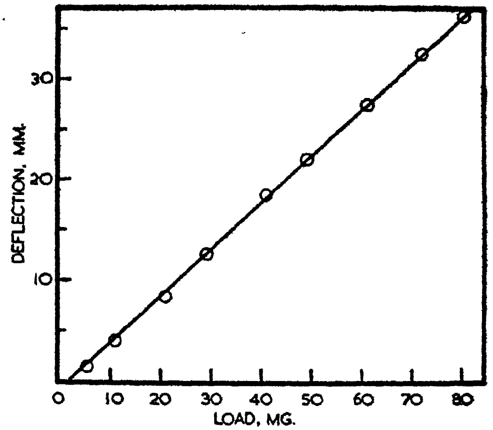

Fig. 6.

Preliminary experiments with the balance showed that readings were difficult to obtain rapidly owing to the large oscillations necessarily set up on applying the load. This difficulty was overcome by the use of artificial damping, accomplished by causing the looped end of the pointer (b, Fig. 2) to dip into thin machine oil contained in the oilbath C. This may be constructed from thin tinplate; alternatively a 10-cm. porcelain boat may be used. The oilbath is filled to about one-half with oil. As is clear from Fig. 5, the use of damping permits weighings to be completed in a few seconds.

Calibration of the scale is carried out by using short pieces of 22 S.W.G. copper wire which have been previously weighed on a balance of normal form. A typical calibration curve is shown in Fig. 6. In practice, the accuracy of the instrument is limited by variations in the distribution of the material in the weighing spoon. If the material is packed against the side of the spoon remote from the fulcrum, it will obviously exert a greater turning moment than if packed against the opposite side of the spoon. This effect is shown in Table I. Each result was obtained by placing a predetermined load in a weighing spoon and repeatedly observing the apparent weight. After each observation, the spoon was gently tapped to redistribute its contents and then replaced on the balance.

TABLE I

REPRODUCIBILITY OF BALANCE

Material	True weight mg.	Observed weight mg.	Max. error mg.
Sucrose powder ..	13·0	13·9, 13·3, 13·1, 12·8, 14·1, 13·3, 13·6, 12·8, 13·9, 13·1	1·1
" " ..	32·2	32·6, 31·8, 32·0, 31·8, 32·6, 32·8, 31·6, 33·6, 33·1, 32·6	1·4
" " ..	51·6	50·7, 52·6, 51·3, 52·1, 51·5, 52·1, 50·7, 51·8, 51·3, 52·4	1·0
Copper wire ..	21·0	21·4, 21·4, 20·8, 21·4, 21·6, 21·6	0·6
" " ..	40·5	40·1, 41·1, 41·3, 41·1, 41·1, 41·1	0·8
" " ..	83·0	82·8, 82·8, 83·4, 83·8, 83·0. 83·0	0·8

The general performance of the balance is shown by the results in Table II, obtained by picking up by shovelling action various amounts of powdered sucrose in a weighing spoon, observing the apparent weight and then determining the true weight on a balance of conventional pattern.

TABLE II

TYPICAL RESULTS IN THE WEIGHING OF POWDERS

True weight mg.	Observed weight mg.	Error mg.
5·0	4·5	− 0·5
13·0	13·4	+ 0·4
22·7	24·0	+ 1·3
30·2	30·4	+ 0·2
51·6	51·6	0·0
77·0	75·6	− 1·4

Though the balance in its present form is not a highly accurate instrument, it is useful for the rapid weighing of reagents for semi-micro preparations, qualitative analysis, etc. Its cost is negligible and its construction involves glass-working of but a rudimentary character. Perhaps the least satisfactory constructional feature is the oilbath method of damping, which hampers the portability of the instrument. This could be overcome by employing a slightly more complicated design, or in all probability by employing electromagnetic means of damping.

CHEMISTRY DEPARTMENT
L.C.C. NORWOOD TECHNICAL INSTITUTE
KNIGHT'S HILL, LONDON, S.E.27

J. T. STOCK
M. A. FILL
December, 1946

Official Appointments

PUBLIC ANALYST APPOINTMENTS

NOTIFICATION of the following appointments of Public Analysts has been received from the Ministry of Health since the last record in THE ANALYST (1947, **72**, 253).

Public Analysts		*Appointments*
DIXON, Frederick (Deputy)	..	City of Manchester.
HERON, Neil (Deputy) ..	..	Borough of Gosport.
PEDEN, Miss J. D. (Deputy)	..	County of Staffordshire.
WILLIAMS, Albert Lester ..	..	Borough of Gosport.

Mr. H. Dedicoat did not take up his appointment as deputy Public Analyst for the County Borough of Leeds (*cf. loc. cit.*).

OFFICIAL AGRICULTURAL ANALYST APPOINTMENTS

NOTIFICATION of the following appointments of Official Agricultural Analysts has been received from the Ministry of Agriculture and Fisheries since the last record in THE ANALYST (1947, **72**, 254).

Official Agricultural Analysts		*Appointments*
HERON, Neil (Deputy) ..	..	County Borough of Liverpool.
WILLIAMS, Albert Lester ..	..	County Borough of Portsmouth.

Ministry of Food

STATUTORY RULES AND ORDERS*

1947—No. 1134. The Fluorine in Food Order, 1947. Dated June 10, 1947. Price 1d.

This Order, forecast in a Note by the Ministry of Food (ANALYST, 1946, **71**, 382;, *prescribes maximum limits for the fluorine content of acidic phosphates used for food purposes and of foods containing acidic phosphates.*

The limits are set forth in a Schedule as follows :

1. Calcium acid phosphate, sodium acid pyrophosphate or any other acidic phosphate: 300 parts per million.
2. Any article of food (not included in items 3 and 4 below) containing calcium acid phosphate, sodium acid pyrophosphate or any other acidic phosphate and intended for use in the composition or preparation of food: 300 parts per million parts of the calcium acid phosphate, sodium acid pyrophosphate or other acidic phosphate present.
3. Baking powders, including golden raising powder: (*a*) 100 parts per million parts of the article of food where such article yields less than 15 per cent. of available carbon dioxide; (*b*) 133 parts per million parts of the article of food where such article yields not less than 15 per cent. of available carbon dioxide.
4. Self-raising flour or any similar mixture (not included in item 3 above) containing a farinaceous substance and an acidic phosphate: 8 parts per million parts of the article of food.

The Order comes into force

(*a*) in respect of items 1 and 2, on October 1, 1947;

(*b*) in respect of items 3 and 4, as regards manufacture for sale, or sales by the manufacturer, on November 1, 1947, as regards sales by wholesale, on January 1, 1948, and as regards sales by retail, on March 1, 1948.

— No. 1310. The Labelling of Food (General Licence) Order, 1947. Dated June 25, 1947. Price 1d.

This Order permits for a limited period the sale of beer brewed in the United Kingdom, free from the labelling requirements of the Labelling of Food Order, 1946. *It ceases to have effect on January* 1, 1949, *in respect of sale by retail and on earlier dates in respect of other sales.*

— No. 1511.—The Soft Drinks (Amendment No. 2) Order, 1947. Dated July 17, 1947. Price 1d.

This Order, from July 20, 1947, *increases the sugar content, and reduces the saccharin content, of carbonated soft drinks, but permits the sale, until October* 20, 1947, *of carbonated soft drinks complying with standards obtaining immediately before this Order came into force.*

In column 5 *of Section* 1 *of the Table in Part II of the First Schedule of the Principal Order, the words* "sugar 3 lb. and saccharin 66 grains" *are replaced by* "sugar 4½ lb. and saccharin 50 grains"; *and the words* "sugar 3 lb. and saccharin 41 grains" *are replaced by* "sugar 4½ lb. and saccharin 25 grains."

— No. 1515.—The Preserves Order, 1947. Dated July 18, 1947. Price 4d.

This Order replaces the Preserves Order, 1944 (*No.* 841), *as amended* (S.R. & O., 1945, Nos. 66 and 196; 1946, Nos. 249, 1485 and 1694; 1947, Nos. 755 and 878). *It frees from price control certain varieties of jam manufactured in the United Kingdom. It applies the labelling provisions of the Order to all imported honey, whether of a "specified brand" or not. It slightly alters the definition of jam, to—*

> "Jam" includes marmalade, jam in the form of jelly, jam made from fruit or vegetables or both, and jam described as "conserve" or "preserve" or by any other name.
>
> "Marmalade" means any jam manufactured from citrus fruit with or without the addition of the juice or pectin of other fruit.

— No. 1867. The Bread (Control and Maximum Prices) Order. Dated August 27, 1947. Price 4d.

This Order, as from August 31, 1947, *replaces the Bread* (*Control and Maximum Prices*) (*No.* 2) *Order,* 1943, *as amended. The restrictions on the quantities of fats and "improvers" used in making bread and on the descriptions and shapes of loaves have been removed.*

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* Obtainable from H.M. Stationery Office. Italics signify changed wording.

In this Order—

"National bread" means bread in the production of which (i) at least 87½ per cent. of the flour used is national flour; and (ii) no ingredients besides national flour have been used, other than one or more of the substances specified in the First Schedule to this Order, *viz., imported flour; oils and fats; water; salt; yeast; improvers of the nature of yeast food, any acid or acidic substances suitable for regulating the acidity of the dough; potato and potato flour; barm.*

"Rye bread" means bread in the production of which at least 25 per cent. of the flour used is rye flour and/or rye meal.

Among the articles to which the provisions of the Order (except for licence to manufacture) do not apply are protein enriched breads, i.e., breads containing not less than 20 *per cent. of protein, the protein being deemed to be equivalent to* 5·7 *times the amount of nitrogen in the bread.*

1947—No. 1891. The Soft Drinks (Amendment No. 3) Order. Dated August 29, 1947. Price 1d.

This Order, as from September 1, 1947, *amends further the Soft Drinks Order,* 1946 (*S.R. & O.*, 1946, *No.* 945; 1947, *Nos.* 478 *and* 1511) *by adding* (*i*) tomato juice, *and* (*j*) points rationed foods (as defined in the Food (Points Rationing) Order, 1947, as amended) *to the exceptions to the definition of "Soft Drink" contained in Article* 1 *of the principal Order.*

British Standards Institution

A FEW copies of the following draft Specifications, issued for comment only, are available to interested members of the Society and may be obtained on application to the Secretary, J. H. Lane, 7–8, Idol Lane, London, E.C.3.

Draft Specification prepared by Technical Committee FCC/4—Solvents.

CJ(FCC)1670—Draft for Ethyl Acetate (Revision of B.S.553).
CJ(FCC)1672—Third Draft for Diacetin (Glyceryl Diacetate).
CJ(FCC)1673—Draft for Hexachlorethane (Revision of B.S.577).
CJ(FCC)1674—Draft for Acetic Acid (Glacial, Technical and Dilute) (Revision of B.S.576 and 578).
CJ(FCC)1675—Draft for Perchlorethylene (Revision).
CJ(FCC)1676—Draft for *Iso*propyl Alcohol (*Iso*propanol).

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Method for Determining the Specific Gravity of Extracts of Jams, Syrups, etc., based on the use of a Density Gradient. J. H. van der Kamer and N. F. Jansen (*Chem. Weekblad*, 1946, **42**, 103–106)—The method is an adaptation of that used by Linderstrøm Lang and others for biological purposes. The principle is that of observing the point of equilibrium of a drop of the material in a column of immiscible liquid having a standardised density gradient. The essential apparatus consists of a 500-ml. measuring cylinder with marks, extending right round the cylinder, at every 5 ml.; a larger cylinder for a water jacket; and a mixer of copper wire (1 mm. diameter) bent to form a flat spiral just fitting into the smaller cylinder (diameter about 4 cm.), the spiral having about 5 complete turns, and the straight stem, rising from its centre, being 45 cm. To prepare the density gradient, two mixtures of petroleum (sp.gr. 0·79) and bromobenzene (sp.gr. 1·49), in the proportions 68·5 : 31·5 and 61·5 : 38·5 by volume, are prepared to give sp.gr. 20°/20° of 1·01 and 1·06, respectively. After 250 ml. of the heavier mixture have been placed in the measuring cylinder, 250 ml. of the lighter mixture are run in from a separating funnel, having the lower end bent at right angles, in such a way that the two layers do not mix. When the temperatures of the two layers have become equalised, the mixer is carefully lowered to the boundary surface, and moved up and down smoothly thirty times over a distance of 50 ml. above and 50 ml. below the boundary layer (*i.e.*, from the 200 to the 300 mark). This movement is repeated thirty times over each of the ranges 150 to 350, 100 to 400, and finally 50 to 450. The gradient so prepared is placed in the water-jacket and allowed to stand overnight. It is then standardised by using a series of copper sulphate solutions as follows:

Sp.gr. 20°C./20°C.	Grams. $CuSO_4$ in 100 g. of solution	Grams of $CuSO_4.5H_2O$ in 100 g. of solution
1·0104	1	1·5643
1·0208	2	3·1286
1·0314	3	4·6928
1·0420	4	6·2571

Intermediate solutions are prepared by mixing equal volumes of the above. A drop of one of the solutions is allowed to fall from a Pasteur pipette under the surface of the gradient, and the point at which the drop comes to rest is noted. The operation is repeated with the other standard solutions, and a standardisation curve is drawn. If the gradient has been properly prepared, this curve is a straight line.

The position of equilibrium of the liquid under examination is then determined similarly, and the specific gravity is read off from the curve. The liquid should have a specific gravity between 1·02 and 1·04; syrups and jams are conveniently tested at a dilution of 10 g. per 100 ml. Duplicate determinations should show a difference of specific gravity not greater than 1 × 10^{-4}, but occasional abnormal results may be recorded owing to the presence of a minute bubble of air or of a fragment of solid matter. The thermal coefficient of expansion of sugar solutions (2·5 × 10^{-4} per 1° C.) is the same as that of the copper sulphate solutions, though considerably different from that of the solvent mixture (9 × 10^{-4} per 1° C.). It follows that the results represent sp.gr. 20°/20° no matter what the actual temperature is, provided that the test and the standardisation are carried out at the same temperature. It is therefore desirable to check the standardisation immediately before and after testing a series of unknowns. If the method is used for butter milk, correct results are obtained if the position of the drop is observed after two or three minutes, but there is a slow change in position with time owing to extraction of the fat by the solvents. It is desirable that the size of the drops should be approximately the same, and this is easily done if two marks are made on the capillary of the Pasteur pipette. The method can not be used for alcoholic solutions, owing to extraction of the alcohol, but it may be applied to beer extracts by using a density gradient of 0·99 to 1·04. The gradient may be used repeatedly over a period of at least one month, and may be "cleaned" from suspended drops by sprinkling a little sand on the surface, so causing the drops to collect at the bottom. G. M.

Estimation of Chicken Manure in Egg Products from their Uric Acid Content. R. E. Duggan (*J. Assoc. Off. Agric. Chem.*, 1946, **29**, 76–84)—Determination of uric acid, which rarely occurs as a normal constituent of food, is suggested as a means of estimating chicken manure in egg products. Much of the visible portion of such manure is removed in the breaking and screening processes, but the soluble and finely divided filth may remain as a serious and hazardous contamination. Several investigators have reported that the uric acid or ammonium urate content of fowl excreta depends upon the diet of the fowl and that, on the dry basis, it ranges from 7·7 to 9 per cent., calculated as uric acid. The most widely used reaction for estimation of uric acid is the development of a blue colour with phosphotungstic acid in the presence of cyanide, a reaction first reported by Folin and Macallum (*J. Biol. Chem.*, 1911, **11**, 265; Analyst, 1912, **37**, 355) and subjected to modifications and refinements by later investigators. The reaction as now used will determine microgram amounts of uric acid.

The reaction cannot be applied directly to egg products because they contain substances that reduce the reagent and produce the blue colour. It appears impractical to separate uric acid from masses of protein matter by precipitation, and it was therefore extracted by means of an immiscible solvent, *viz.*, quinoline, in which it is soluble to the extent of 1·13 g. per 100 ml. Uric acid solutions as extracted in this procedure are unstable, and the analysis must be finished on the same day as begun, since low, erratic results are obtained if the extracts in quinoline or sodium phosphate solution are stored overnight, even at low temperatures.

Method—Centrifuge 100 g. of liquid eggs in a 250-ml. centrifuge bottle at not less than 2000 r.p.m. for at least 5 min. Weigh the decanted supernatant liquid in a tared beaker, and leave the sediment and heavy egg material adhering firmly to the bottom of the bottle. Treat the sediment (in the original centrifuge bottle) and 10 g. of the decanted liquid (in another centrifuge bottle) separately by the following procedure.

Add 100 ml. of dilute sulphuric acid of *p*H 2·5, emulsify by vigorous shaking, add 25 ml. of quinoline, shake vigorously for 1 min., add another 25 ml. of quinoline, shake vigorously for 3 min., and centrifuge at 2000 r.p.m. for 5 min. Stop the centrifuge and invert the bottle several times to break up the lower layer, but do not shake. Again centrifuge for 5 min. and allow the machine to stop of its own accord. The bottle will now contain three layers, *viz.*, the top aqueous layer, the middle precipitated egg layer, and the bottom quinoline layer. Tilt the bottle so that the uppermost aqueous layer touches the lowermost quinoline layer (the intermediate layer acting as a valve) and pour the two layers into a 250-ml. separating funnel, thereby leaving the precipitated egg layer in the bottle. If only a part of the quinoline layer has separated, it will be necessary to shake and re-centrifuge the precipitated layer until all the quinoline has been separated. Allow the liquid in the separating funnel to settle, and filter the quinoline layer into a dry, 110-ml. flask through rapid, fluted filter paper.

Drain the aqueous layer into the centrifuge bottle containing the corresponding precipitated egg layer, add 25 ml. of quinoline, shake for 3 min., and centrifuge, separate and filter as before. Repeat the extraction a third time with 20 ml. of quinoline. Finally, shake and centrifuge the precipitated egg layer until no more quinoline separates.

Wash the filter through which the quinoline extracts have been filtered with small portions of ether until the combined volume of the extracts and the ether washings is 110 ml. To a 50-ml. aliquot of the thoroughly mixed liquid in a separating funnel add an equal volume of ether. Extract successively with a 25-ml. and a 10-ml. portion of 2 per cent. disodium phosphate (Na_2HPO_4) solution, run each aqueous extract into a separating funnel containing 25 ml. of benzene and, after washing with the benzene, combine the aqueous extracts in a 50-ml. flask and dilute to that volume with water.

Pipette exactly 5 ml. of the well-mixed liquid into a 50-ml., glass-stoppered Pyrex graduated cylinder, and add from burettes exactly 5 ml. of a freshly prepared, 5 per cent. sodium cyanide solution and 1 ml. of the arsenophosphotungstate reagent (*infra*). Mix by one inversion and place the cylinder immediately in boiling water for 3 min. Dilute to 50 ml. with water and mix well. It is not necessary to cool the solution.

Determine immediately the amount of colour developed, using a 50-ml. cell in a photometer fitted with a filter transmitting at 650 mμ. Make a blank determination by extracting the sediment and liquid from clean eggs in the same manner and correct the photometric readings of the liquid and the sediment by deducting their respective blank values. Calculate the uric acid content of each portion by means of a standard curve. The total uric acid content of the sample in mg. per 100 g. is given by

$$\left[\left(a + \frac{bc}{10}\right) \times \frac{v}{5} \times \frac{110}{50}\right]/1000$$

where a is the uric acid (μg.) in the sediment, b is the uric acid (μg.) in 10 g. of the decanted liquid, c is the weight in grams of the decanted liquid, and v is the total volume (ml.) of the solution of which 5 ml. were used to develop the colour.

To construct the standard curve, dilute the standard uric acid solution (*infra*) to a concentration of 0·005 mg. of uric acid per ml. Measure accurately from a micro-burette 5-, 10-, 15-, and 20-μg. portions into 50-ml., glass-stoppered, Pyrex, graduated cylinders and add enough water to each portion to make 5 ml. Add exactly 5 ml. of freshly prepared, 5 per cent. sodium cyanide solution and 1 ml. of the arsenophosphotungstate colour reagent and heat the cylinders immediately in boiling water for exactly 3 min. Dilute to 50 ml. with water and immediately determine the colour as already described for the sample. Determine the reagent blank value using 5 ml. of water in place of the uric acid solution. A new standard curve must be constructed for each batch of the colour reagent and for each different bottle of sodium cyanide.

To prepare the arsenophosphotungstic colour reagent (Benedict, *J. Biol. Chem.*, 1922, **51**, 187), dissolve 100 g. of sodium tungstate in 600 ml. of water and add, in order, 50 g. of arsenic pentoxide, 25 ml. of 85 per cent. phosphoric acid, and 20 ml. of concentrated hydrochloric acid. Boil vigorously for 20 min., cool, and dilute to 1 litre.

To prepare the standard uric acid solution (Folin, *Ibid.*, 1930, **86**, 179) place exactly 1 g. of uric acid, dried to constant weight at 100° C., in a litre flask and pour on it 0·6 g. of lithium carbonate dissolved in 150 ml. of warm water, swirl until a clear solution is obtained, and cool immediately. Add 20 ml. of 40 per cent. formaldehyde, half fill the flask with water, add 25 ml. of *N* sulphuric acid, and dilute to 1 litre. Fresh, working, standard solutions should be prepared daily by dilution of this stock solution, which is stable for 2·5 years.

Blank determinations on seven samples of clean eggs gave an average uric acid equivalent in 2 to 3 g. of clean sediment of 19·8 ± 4 μg. and in 10 g. of clean egg liquid of 41·8 ± 2 μg. No significant variation in these blank values was found with eggs from a wide geographical area and no change occurred after refrigerated storage of the samples for a year.

The method is satisfactorily precise, is applicable to reconstituted dried egg, and is capable of estimating less than 1 part of uric acid in 100,000 parts of egg, corresponding to about 35 mg. of fresh manure per 100 g. Six samples of chicken manure were found to have an average of 67·1 per cent. of moisture and 26·5 per cent. of water-insoluble matter. Air-dried manure was found to contain 10 per cent. of moisture and 66 per cent. of water-insoluble matter. After drying the samples at 100° C., the average uric acid content was found to be 9·2 per cent. A. O. J.

Determination of DDT Deposits on Fruits, Vegetables, and Vegetation. R. H. Carter and P. E. Hubanks (*J. Assoc. Off. Agric. Chem.*, 1946, **29**, 112–114)—Recent methods for the determination of small amounts of DDT in a variety of materials include those based upon the determination of chlorine after decomposition of the DDT by heat, reduction by metallic sodium, and dehydrohalogenation by reaction with alcoholic potassium hydroxide. The dehydrochlorination method determines only one of the chlorine atoms in the molecule, whereas the sodium reduction method determines five chlorine atoms, *i.e.*, 50 per cent. of the DDT. Determinations of chlorine in spray residues by both these methods make it possible to judge to what extent the DDT has been decomposed by weather and other conditions, since the dehydrochlorinated product does not react with alcoholic potassium hydroxide to give the chloride ion. This information is of value because the dehydro halogenated product is relatively non-toxic to insects.

Methods—Extract a weighed amount of fruit, vegetables or vegetation, or a measured area of leaf surface, with a measured amount of a suitable solvent, *e.g.*, benzene or pentane, in a closed container with mechanical agitation for 15 to 30 min. according to the nature of the sample. Remove the solvent from an aliquot of the extract on the steam-bath.

In the alcoholic potassium hydroxide method, boil the residue under refluxing conditions with 50 ml. of 0·1 *N* alcoholic potassium hydroxide for 5 min., cool, dilute with water, acidify with nitric acid, and determine the chloride ion by the Volhard procedure with 0·1 *N* or 0·05 *N* silver nitrate.

In the metallic sodium reduction method, add to the extract 25 to 50 ml. of *iso*propanol and 2 to 4 g. of metallic sodium, and heat the mixture on the hot-plate under refluxing conditions for 2 hr. After cooling, dilute the reaction mixture with water, acidify with nitric acid, and determine the chloride ion as before.

Blank determinations should be made to allow for the presence of extractable halide in the sample. Sulphur and lime-sulphur residues interfere with both methods, but the interference can be prevented by addition of 10 ml. of 30 per cent. hydrogen peroxide to the alkaline solution after dilution, and allowing it to stand for a few minutes before acidifying it. Precipitates formed when the solution is acidified should be filtered off before the silver nitrate solution is added. Coloured solutions may be cleared by addition of chlorine-free decolorising carbon, and filtration. The precision of the method is satisfactory. A. O. J.

Raw Sugar: Sampling, Mixing, and Testing. E. F. Kenney (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 684–688)—U.S. Customs Regulations of 1937 provided that, with imported raw sugar in bags, 100 per cent. of the bags should be sampled with a trier: the samples so obtained were to be mixed for a test of direct polarisation. In 1942, the conservation of jute bags became desirable, and experiments were made with triers that would cause less damage to the bags. The three triers used gave almost identical standard deviations of about 1·04° S. The Canadian trier, although causing less damage to the bags, was unsuitable for hard, gummy sugar, and the modified Customs trier was no better than the standard Customs trier. Accordingly, the latter was standardised, and, using the formula $n = t^2 \sigma^2/E^2$, where n is the number of bags to be sampled, t is Student's t for the 0·99 level of probability, σ the standard deviation, and E the maximum permissible sampling error, a table was drawn up relating E to n. For maximum sampling errors of 0·5, 0·2, 0·1, 0·05, 0·02, 0·01° S, the corresponding numbers of bags to be sampled are 32, 201, 803, 3217, 20,073, and 80,293, respectively. An average cargo being 20,000 bags, a sampling ratio of 1 bag in 7 was tested experimentally and found to be satisfactory. In a set of five cargoes, the maximum difference between the revenue as estimated from 100 per cent. sampling and from 1 bag in 7 sampling was $68 in a total of $124,000.

Experimental work on the mixing of samples led to the development of a so-called box and screen method (described in Circular Letter 2416 of Bureau of Customs, U.S. Treasury Dept., 1943) in which a 0·375-in. mesh wire screen was fitted over a 4-box receiver. There was little difference in mixing precision between this method and the older hand method. K. A. B.

Use of Low-temperature Crystallisation in the Determination of Component Acids of Liquid Fats. I. Fats in which Oleic and Linoleic Acids are Major Components. T. P. Hilditch and J. P. Riley. II. Fats which contain Linolenic as well as Linoleic and Oleic Acids. F. D. Gunstone and T. P. Hilditch. III. Fats which contain Elaeostearic as well as Linoleic and Oleic Acids. T. P. Hilditch and J. P. Riley (*J. Soc. Chem. Ind.*, 1945, **64**, 204–207; 1946, **65**, 8–13, and 74–81)—Preliminary separation of mixed fatty acids by crystallisation from solvents at low temperature (*cf.* Brown *et al.*, *Chem. Rev.*, 1941, **29**, 333) is preferable to lead salt separation when the proportion of saturated acids is low (*i.e.*, approx. 10 per cent. or less of the total acids). The low temperature is obtained by addition of solid carbon dioxide to an alcohol bath. Fats in groups I to III (*loc. cit.*) which were examined were:

Group I. (*a*) Sunflower seed, (*b*) sesamé, (*c*) groundnut (probably Indian), and (*d*) Argentine groundnut oils.

Group II. (*e*) Linseed, (*f*) wheat germ, (*g*) Ceylon rubber seed, and (*h*) Nigerian rubber seed oils.

Group III. (*i*) Commercial tung, (*j*) American tung, (*k*) neou, (*l*) essang, and (*m*) krobanko oils.

In group I, conversion of the fractionally crystallised fatty acids into methyl esters and subsequent fractionation of each portion of esters are preferable to ester-fractionation of the total fatty acids. In group II, the mixed fatty acids are first separated into several groups by crystallisation from appropriate solvents at low temperature, and the proportions of linolenic, linoleic, oleic and saturated acids in each group determined spectrographically after isomerisation with alkali (*cf.* Hilditch, Morton, and Riley; ANALYST, 1945, **70**, 67). Palmitic and other saturated acids may be determined by fractional distillation of the completely hydrogenated methyl esters of the total mixed fatty acids, or from direct ester-fractionation of the methyl esters of each group of the mixed acids obtained by low-temperature crystallisation. The accuracy of the determination depends largely on the degree to which the mixed fatty acids are resolved into (i) portions rich in oleic and/or saturated acids (with only minor amounts of linoleic and linolenic acid), and (ii) *vice versa*. III. The mixed fatty acids are first separated into several groups by crystallisation from acetone, and then from light petroleum, at low temperature. Each group is analysed spectrographically before and after isomerisation with alkali to determine the proportion of α-elaeostearic and linoleic and, if present, linolenic acids. On the mixed fatty acids, the saturated fatty acids are determined by the Bertram method, palmitic acid is determined by fractionation of the hydrogenated mixed esters, and oleic acid by difference. Preliminary low-temperature resolution into several groups is essential, as with fatty acids very rich in elaeostearic acid spectrographic analysis of the total fatty acids may lead to errors in the determined proportion of linoleic and other acids. Working details are given. Component acids calculated as percentage weight, excluding unsaponifiable matter follow; also the unsaponifiable matter in total fatty matter.

	Group I				Group II				Group III				
	(*a*)	(*b*)	(*c*)	(*d*)	(*e*)	(*f*)	(*g*)	(*h*)	(*i*)	(*j*)	(*k*)	(*l*)	(*m*)
Myristic ..	Trace	0·1	0·5	0·4	—	—	—	—	—	1	—	—	—
Palmitic ..	6·4	8·2	8·0	11·4	7	16	11	9	4	4·5	12	9·5	12
Stearic	3·2	3·6	4·4	2·8	8	6	12	10	—	—	2	1	7
Arachidic ..	0·9	1·1	—	—	1	—	1	1	—	—	—	—	—
Saturated C_{20}, C_{22}, C_{24} ..	—	—	6·6	7·3	—	—	—	—	—	—	—	—	—
Hexadecenoic ..	0·4	0·5	1·7	2·4	—	—	—	—	—	—	—	—	—
Oleic	21·6	45·3	52·5	42·3	13	12	17	20	9	4	40	9·5	21
Linoleic	67·5	41·2	26·3	33·4	17	57	35	39	10	8·5	15	26	23
Linolenic ..	—	—	—	—	54	9	24	21	—	—	—	—	—
α-Elaeostearic ..	—	—	—	—	—	—	—	—	77	82	31	54	16
Acids yielding resinous compound ..	—	—	—	—	—	—	—	—	—	—	—	—	21
Unsap. matter ..	0·9	1·6	0·2	0·1	0·9	0·5	0·5	0·9	(——— not given ———)				

Results for groups II and III are given to 0·5 per cent. as the accuracy of these determinations is less than for group I. Earlier results of determination by lead salt separation, spectrographic analysis, and thiocyanogen value are given, with references.

E. B. D.

Biochemical

Polarographic Determination of Oestrone [in Urine]. **O. Bjornson and M. Ottesen** (*Quart. J. Pharm.*, 1946, **19**, 519–526)—Like other 17-ketosteroids, oestrone is not reduced at the dropping mercury electrode, but its derivative with Girard reagent T, trimethylacetylhydrazide ammonium chloride, gives rise to a polarographic wave at −1·38 to −1·40 v. (*versus* the saturated calomel electrode), which can be used analytically in solutions containing 2 to 100 μg. per ml.

The determination of oestrone in urine samples requires an initial extraction of the hormone to free it from other reducible substances. The method for the extraction and determination described here follows that of Wolfe, Hershberg, and Fieser (*J. Biol. Chem.*, 1941, **140**, 215). This extraction permits the determination of both free and combined oestrone in the same sample.

Procedure—Extract 15 ml. of unhydrolysed urine with two 15-ml. portions of ether, reserving the extracted urine sample for a determination of the combined oestrone. Wash the ether layer with 4 ml. of 10 per cent. sodium carbonate solution, and then extract the oestrone with two 15-ml. portions of *N* sodium hydroxide solution. Acidify the aqueous solution with 10 ml. of 4·5 *N* sulphuric acid, and extract the oestrone with one 15-ml. portion and one 7-ml. portion of chloroform. After washing the chloroform extract with 30 ml. of 0·67 per cent. sodium carbonate, evaporate to dryness, re-dissolve the residue in 1 ml. of chloroform, transfer it to a test tube by means of 0·5 ml. of chloroform, and re-evaporate it to dryness. Add to the residue 0·02 ml. of a solution containing 100 mg. of Girard reagent T in 1 ml. of glacial acetic acid, stopper and tie the tube, and heat it in boiling water for exactly 2 min. Cool the mixture immediately, and add in succession 0·48 ml. of re-distilled water, 0·50 ml. of 0·5 *N* ammonium chloride, and 1·0 ml. of 0·2 *N* sodium hydroxide. After removing dissolved oxygen by bubbling nitrogen through the solution for 5 min., examine it in the polarograph.

The combined oestrone is hydrolysed by boiling the urine, after extraction, with 3 ml. of 10 per cent. sulphuric acid for 2 min. It is then extracted and determined in the same way as free oestrone.

Interfering substances are introduced by the use of impure reagents, and the accuracy of the method depends on the use of freshly purified reagents. Chloroform should be re-distilled, and the anhydrous acetic acid, prepared from acetic anhydride, should be fractionated. The Girard T reagent should be freshly crystallised from absolute alcohol.

J. G. W.

Solid Polyethylene Glycols [Carbowax Compounds]. Quantitative Determination in Biological Materials. C. B. Shaffer and F. H. Critchfield (*Anal. Chem.*, 1947, **19**, 32–34)—The lower members of the polyethylene glycols, *viz.*, diethylene glycol and triethylene glycol, are familiar industrial chemicals, but recently members of higher molecular weight have become available, not as separate compounds but as mixtures of individual polymers. Four liquid and five solids are now obtainable, the solids being known as Carbowax compounds (Carbide and Carbon Chemicals Corporation). Each product represents a range of molecular weights, the average of which (with the exception of Carbowax compound 1500) is used to characterise that particular member of the series. Carbowax compound 1500 is a blend of approximately equal weights of polyethylene glycol 300 and Carbowax compound 1540. The liquid compounds are thus known as polyethylene glycols 200, 300, 400, and 600, and the solids as Carbowax compounds 1000, 1500, 1540, 4000, and 6000. Their pharmacology has been studied. Their acute toxicity by oral or external administration is low, but little is yet known about their absorption, metabolism, and excretion from the body, mainly owing to the lack of a suitable method for their detection and determination in blood and excreta. Their low chemical reactivity and mixed composition render development of a method difficult, and the only reaction of any practical analytical significance is the formation in acid solution of highly insoluble complexes with such heteropoly-inorganic acids as phosphomolybdic and silicotungstic acids in presence of the cation of a heavy metal, *e.g.*, barium. With these reactions a colorimetric method for whole blood and plasma and a gravimetric method for urine have been devised.

Preparation of sample—Filtrates of whole blood or plasma that are completely free from protein are suitable for the analysis if they have not been prepared with tungstic or molybdic acids. Preference has been given to the precipitation of the plasma proteins with zinc sulphate and barium hydroxide (Somogyi, *J. Biol. Chem.*, 1945, **160**, 69). Sulphate interferes with the gravimetric procedure, and even in the colorimetric method it is desirable to remove it. In spite of careful balancing of the zinc sulphate and barium hydroxide solutions, the filtrates thus prepared invariably contain excess of sulphate, which must be removed as barium sulphate before precipitation of the polyglycol. In the preparation of urine for the gravimetric method, Fiske's ferric acetate treatment as described by Hoffman (*J. Biol. Chem.*, 1931, **93**, 787) has been found suitable for the removal, from normal urine, of interfering substances with the exception of sulphates. For the removal of sulphates, an aliquot portion of the filtrate is diluted to 50 ml., 4 ml. of concentrated hydrochloric acid are added, and the sulphates are hydrolysed and precipitated according to Folin's directions for the determination of total sulphates in urine (Peters and Van Slyke, "*Quantitative Clinical Chemistry*," Vol. II, "Methods," pp. 771, 893; Baltimore, Williams and Wilkins Co., 1932). After removal of the barium sulphate by filtration, the filtrate is ready for precipitation of the polyglycol contained therein.

Gravimetric method—Place the sample freed from interfering materials in a 600-ml. beaker, add 10 ml. of diluted hydrochloric acid (1 + 4) and 10 ml. of 10 per cent. barium chloride solution. For ease of subsequent filtration it is desirable that the quantity of polyglycol present be less than 0·1 g. With urine treated as described (*supra*) enough of these reagents are already present and more need not be added. Dilute the liquid to 250 or 300 ml. and heat it to the boiling point. Precipitate the polyglycol with 10 ml. of silicotungstic acid solution (10 g. of silicotungstic acid, $4H_2O.SiO_2.12WO_3.22H_2O$, dissolved in a little water, then neutralised with 10 per cent. sodium hydroxide solution to methyl red, and diluted to 100 ml.) added slowly from a pipette, boil the liquid for a few seconds to coagulate the precipitate, and set the cooled mixture aside for 8 to 12 hr. Collect the precipitate with suction in a tared Gooch crucible, wash it thoroughly with not less than 200 ml. of water, dry at 110° C. for several hours, and finally ignite in a muffle furnace at 700° C. for successive 30-min. periods until its weight is constant. The temperature is important as it is the optimal temperature to minimise both incomplete dehydration of the silicon oxide and volatilisation of tungstic oxide. In filtering the precipitate, the liquid must not be allowed to run dry. From the weight of ignited residue obtained, which consists of the mixed oxides of silicon, tungsten, and barium, the weight of polyglycol originally present in the sample is calculated by means of an empirical factor determined from known quantities of the polyglycol by the same method.

Colorimetric method—Place 10 ml. of a protein-free filtrate of blood plasma, which has also been freed from sulphate, in a 15-ml., graduated centrifuge tube and add, in the order given, 1 ml. of diluted hydrochloric acid (1 + 4), 1 ml. of 10 per cent. barium chloride solution, and 1 ml. of 10 per cent.

phosphomolybdic acid solution, stirring the mixture after each addition with a thin glass rod. Allow the tube to stand for at least 1 hr. Centrifuge at 2500 r.p.m. for 10 min. and slowly and carefully draw off the supernatant liquid without disturbing the characteristic flocculent, greenish precipitate formed by interaction of the polyglycol with the reagents. For this purpose, a capillary device such as the one described for washing calcium precipitates (Peters and Van Slyke, *op. cit.*, *loc. cit.*) is useful. Wash the precipitate and tube with two portions of 0·1 *N* hydrochloric acid, allowing 3 ml. to flow slowly down the sides of the tube, then stirring the precipitate with the glass rod, and finally rinsing down the rod and the walls of the tube with 7 ml. of distilled water. Repeat the centrifuging and washing. Draw off the liquid, transfer the precipitate quantitatively to a 100-ml. Kjeldahl flask with the minimum amount of water, add 3 ml. of concentrated sulphuric acid, and digest with nitric and perchloric acid in the usual way. Cool, dilute with 20 ml. of water, neutralise to phenolphthalein with 40 per cent. sodium hydroxide solution, make just acid with a few drops of sulphuric acid, and finally make the volume up to 100 ml.

To determine molybdenum in this solution add to a 10-ml. aliquot in an Evelyn colorimeter tube 5 ml. of a phenylhydrazine solution prepared by diluting 3 ml. of concentrated sulphuric acid to 60 ml. with water, adding 3 ml. of freshly distilled phenylhydrazine, shaking well and diluting to 100 ml., and mix the liquids by swirling. Close the tube by means of a rubber stopper pierced by a fine capillary tube and place it in the hot water-bath at 81° ± 2° C. for exactly 15 min.; then remove it, and allow it to cool to room temperature. Read the transmission of the solution in the colorimeter, using filter 490 and setting the galvanometer at 100 with a blank determination carried through all the steps of the process. Read the polyglycol content of the solution from a standard curve prepared from known quantities treated exactly as the sample. The curve showing concentration against extinction is linear. The useful range of the method is 0·05 to 1·0 mg. of polyglycol at a minimum concentration of 0·01 mg. per ml.

The following gravimetric factors, defining the amount of Carbowax compound represented by unit weight of the residue, were found—Carbowax compound 6000, 0·2712; 4000, 0·2808; 1540, 0·2487; 1000, 0·2544. The proportions of the components of the Carbowax compounds used industrially are varied to maintain constant physical properties, thereby causing corresponding variations in the gravimetric factor. With ten consecutive production batches of Carbowax compound 4000 the residues in the gravimetric procedure ranged from 3·56 to 3·78 (mean 3·661 ± 0·004) mg. per mg. of desiccated polyglycol. A. O. J.

Organic

New Method for the Detection and Determination of Monochloroacetic Acid. L. L. Ramsey and W. I. Patterson (*J. Assoc. Off. Agric. Chem.*, 1946, **29**, 100–111)—When monochloroacetic acid is heated with an excess of pyridine at about 60° C., a well-defined crystalline mass is precipitated (Gerichten, *Ber.*, 1882, **15**, 1251). This serves as a qualitative test and, if confirmation is desired, pyridine betaine picrate may be prepared from the precipitate and its m.p. determined. For quantitative purposes the precipitation is made under controlled conditions and the purified and dried product is weighed. The precipitate is apparently a mixture of neutral pyridine betaine hydrochloride (N-carboxymethyl pyridinium chloride) and basic pyridine betaine hydrochloride (the double salt of N-carboxymethyl pyridinium chloride and N-carboxymethyl pyridinium hydroxide). The amount of monochloroacetic acid corresponding to the weight of precipitate formed under the experimental conditions is ascertained by means of a standard curve.

Construction of the standard curve—To 10-, 20-, 30-, 40-, and 50-mg. samples of monochloroacetic acid in dry, tared centrifuge tubes add 0·5 ml. of pyridine and swirl the tubes gently to promote solution. Heat the tubes, stoppered with corks lined with tinfoil, in a constant temperature water-bath at 60° ± 2° C. for 2 hr., and then allow them to stand at room temperature overnight. Break up the crystalline mass with a spatula, remove the excess of pyridine by evaporation under reduced pressure, and, when all the liquid has been removed, add 0·5 ml. of pyridine, mix well, centrifuge, and remove the supernatant liquid by decantation. Add 5 ml. of ether, shake well, centrifuge, and decant. Add 1 to 3 ml. of absolute alcohol according to the amount of precipitate, and heat the tube in a water- or steam-bath until the precipitate has dissolved, avoiding superheating and loss of alcohol. Cool in an ice-bath, add about 10 ml. of ether, mix well, and allow to stand in the ice-bath for about 10 min. then centrifuge, pour off the supernatant liquid, and wash the precipitate once with about 5 ml. of ether. Spread the precipitate on the sides of the tube and remove most of the ether by placing the tube in a water-bath at 40° to 50° C. for 2 or 3 min. Finally, dry the precipitate at 100° C. for 15 min., cool in air, and weigh. Plot the weights of the betaine precipitates obtained against the weights of monochloroacetic acid taken.

Quantitative method for beverages and fruit juices—Place 100 ml. of the sample (diluted with an equal volume of water if it is a syrup) in a large continuous liquid extractor or in two lactic acid extractors. If the sample contains less than 200 p.p.m. of monochloroacetic acid, a larger portion should be taken. Add 2 or 3 ml. of diluted sulphuric acid (1 + 1) and extract with ether for 2 hr. When emulsions interfere with the extraction centrifuge the sample with a little Filter-Cel before extraction.

To the ether extract in a separating funnel add a small piece of universal indicator paper and enough saturated sodium bicarbonate solution (5 ml.) to make the aqueous layer alkaline (*p*H 7 to 8) after vigorous shaking. Add enough water to make the total volume of the aqueous layer about 10 ml. and shake again. Draw off the aqueous layer into a small separating funnel and wash the ether layer with two successive, 5-ml. portions of water. Wash the combined extracts and washings once with 5 to 10 ml. of ether, add about 1 ml. of diluted sulphuric acid (1 + 1) in excess of the amount required to neutralise the alkaline solution, and extract the acidified liquid with four, 25-ml. portions of ether. Wash the combined ether extracts once with 1 or 2 ml. of water and allow the ether solution to stand for a few min., after drawing off most of the water, until the separation is as complete as possible. Pour the ether through a folded filter paper into a 200-ml. flask, and wash the separating funnel and filter with two 10-ml. portions of ether.

To the ether filtrate add 0·5 ml. of pyridine and a small glass bead, mix, and remove the ether on a steam-bath. Reduce the volume by evaporation to 2 or 3 ml., transfer the liquid immediately by means of an eye-dropper pipette into a 15-ml. centrifuge tube and wash the flask successively with 2-ml., 1-ml., and 1-ml. portions of ether. Reduce the volume of the liquid in the tube to about 0·3 ml. Add enough pyridine to increase the volume to about 0·5 ml. and place the tube in a constant temperature bath at 60° ± 2° C. Proceed as in the construction of the standard curve. For an approximate value, multiply the weight of the precipitate by 0·67 to obtain the weight of monochloroacetic acid. For a more precise result, read the quantity of acid from the standard curve.

When a large amount of another ether-soluble acid such as benzoic acid is present, it may be necessary to carry out the reaction in more than 0·5 ml. of pyridine. Appropriate controls should then be run. Such a condition is unusual, and if benzoic acid were present a voluminous precipitate would be observed when the volume of liquid in the reaction tube was reduced by evaporation of the ether.

Qualitative method for beverages and fruit juices—Proceed as in the quantitative method with the following changes. Extraction in the liquid extractor for 1 hr. is sufficient or the sample may be extracted in a separating funnel with an equal volume of ether. One extraction of the ether solution with sodium bicarbonate solution and two extractions of the acidified bicarbonate extract are sufficient. Transference of liquids need not be quantitative, and the water-bath temperature may range from 55° to 65° C., and when crystals appear the heating need not be continued. If crystals do not appear during the 2-hr. heating period continue as directed in the quantitative procedure, omitting the standing of the precipitate overnight before evaporation of the pyridine under reduced pressure. Formation of characteristic crystals in the reaction mixture constitutes a positive test for monochloroacetic acid, and their appearance serves to distinguish tentatively between the betaine mixture derived from monochloroacetic acid and the product from bromoacetic acid and iodoacetic acid. If all the residue in the tube dissolves at the stage when fresh pyridine is added, the test is negative, but if a precipitate forms during the evaporation stage and is not dissolved by the fresh pyridine, the test is positive.

If it is desired to confirm the qualitative test by identifying the precipitate formed as a betaine or mixture of betaines, dissolve 50 mg. or more of the precipitate in the least amount of hot, 95 per cent. alcohol and add to it 1·5 times its weight of picric acid also dissolved in the least amount of hot, 95 per cent. alcohol. If crystallisation does not occur as the liquid cools to room temperature, concentrate the liquid as described in the quantitative procedure. Centrifuge, decant the mother liquor, wash the residue once with 1 or 2 ml. of absolute alcohol and twice with 2-ml. or 3-ml. portions of ether. Dry the crystals between filter paper and determine the m.p. (142° to 143° C.). Recrystallisation from alcohol may be necessary. Identifying this compound proves that the precipitate is a betaine or mixture of betaines, but bromoacetic acid and iodoacetic acid will give the same pyridine betaine picrate as monochloroacetic acid.

The method which has been described is specific for acids containing a reactive α-halogen atom, but the details of a procedure for determining monochloroacetic acid in the presence of other such acids have not been studied. Moderate amounts (20 mg.) of saccharin, benzoic acid, dichloroacetic acid, and trichloroacetic acid do not interfere; recoveries of monochloroacetic acid (40 mg.) in their presence range from 99 to 105 per cent. For the evaporation of pyridine under reduced pressure, the apparatus described by Fieser (*"Experiments in Organic Chemistry,"* 2nd Ed., 1941) is recommended. A. O. J.

Identification of Crystalline Organic Compounds by means of X-Ray Analysis. J. J. de Lange and J. P. W. Houtman (*Rec. Trav. Chim.*, 1946, **65**, 891–896)—It has been found possible to identify the 2 : 4-dinitrophenylhydrazones of several carbonyl compounds and the 3 : 5-dinitrobenzoic acid esters of some alcohols by comparing the X-ray patterns, Astbury's recording technique (Astbury and Woods, *Proc. Roy. Soc.*, 1934, **232A**, 333) being used.

The X-ray diagrams of the hydrazones of *cyclo*hexanone, 2-methyl*cyclo*pentanone and 3-methyl*cyclo*hexanone differ clearly from one another. The pattern of the hydrazone of acetaldehyde is distinctly different from that of the hydrazones of *iso*-butyraldehyde and methyl ethyl ketone. With acetaldehyde and acetone there is a marked difference between the patterns, but in the positions corresponding to the most characteristic lines of the acetone compound there are also lines in the diagram of the acetaldehyde compound, so that it is impossible to detect with certainty a small proportion of acetone in presence of a large proportion of acetaldehyde, and *vice versa*. The diagrams of the hydrazones of acetone and *iso*-butyraldehyde differ considerably from one another, as do those of acetone and methyl ethyl ketone; the detection of small quantities of *iso*-butyraldehyde in presence of much methyl ethyl ketone might be difficult. There is no possibility of confusion between the patterns of the phenylhydrazones and the hydrazine itself.

Considerable differences in melting point of the 2 : 4-dinitrophenylhydrazone of acetaldehyde have been reported. These are shown to be due to irremovable impurities and not to different crystal structures.

The 3 : 5-dinitrobenzoates of 3-methyl*cyclo*hexanol (*cis* and *trans*) and of ethanol showed a great difference in X-ray pattern.

On distillation of mixtures of 3-methyl*cyclo*hexanone and 3-methyl*cyclo*hexanol at atmospheric pressure in a Vigreux column (2 metres long, 18 theoretical plates) the higher-boiling residue contains 8 per cent. of a ketone, determined titrimetrically with hydroxylamine hydrochloride. X-ray examination of the 2 : 4-dinitrophenylhydrazone proved this ketone to be residual 3-methyl*cyclo*hexanone.

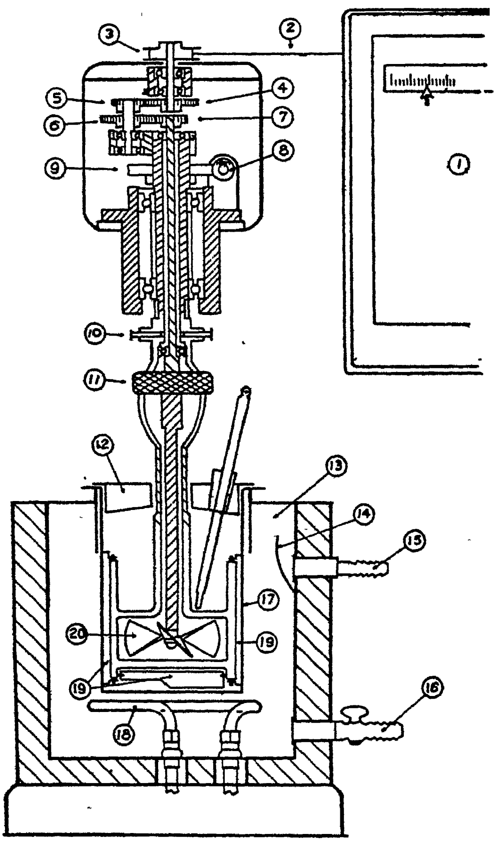
In the distillation of the residue from the pyrolysis of *iso*-butyl acetate to form *iso*-butene, there was obtained a fraction, b.p. 68° to 72° C., with a feebly acrid smell. Study of the phenylhydrazones showed that this product was principally acetone, with possibly some acetaldehyde; another compound with higher b.p. and refractive index, but containing no carbonyl group, is also present. The X-ray method undoubtedly gave quicker results than qualitative analysis in the investigation of this distillation residue. E. M. P.

X-Ray Identification and Crystallography of Aldehydes and Ketones as the 2 : 4-Dinitrophenylhydrazones. G. L. Clark, W. I. Kaye, and T. D. Parks (*Ing. Eng. Chem., Anal. Ed.*, 1946, **18**, 310–313)—The existing methods of identification of aldehdsey and ketones tend to be lengthy and difficult, and for such work the authors advocate the use of X-ray diffraction methods. In this paper, X-ray diffraction techniques are used to characterise 12 different crystalline aldehyde-2 : 4-dinitrophenylhydrazones from 6 different aldehydes, and 16 ketone derivatives from 15 ketones. Several of the aldehyde and ketone derivatives are polymorphic. Weissenberg, rotation, oscillation, and powder patterns are used. The study involves the practical reciprocal lattice interpretation of the powder pattern of a triclinic crystal. Tables listing the three strongest powder diffraction lines and the innermost line make possible the identification of 28 hydrazones.

A micro-method of qualitative analysis for pure and mixed carbonyl compounds is also described. 0·1 Mg. is the minimum amount necessary for analysis, although single crystals weighing less than 1 μg. may be identified. The qualitative micro-method is extended to quantitative analysis. This is achieved by accurate measurement of line densities of known mixtures of the aldehyde derivative with sodium fluoride as an internal standard. Standard intensity ratios are given for a number of aldehyde derivatives. E. G. S.

Recording Viscometer for Starches. C. C. Kesler and W. G. Bechtel (*Anal. Chem.*, 1947, **19**, 16–21)—The most important criterion of the suitability of starches for industrial uses is the apparent viscosity of the paste formed when the starch is cooked with water at a definite concentration. These pastes, which are mixtures of swollen granules and fragments of granules dispersed in a solution of molecules leached from the granules (Katz *et al.*, *Trans. Faraday Soc.*, 1938, **34**, 1258), appear to exhibit true viscosity only when the concentration is low, *e.g.*, below 2 per cent. for maize starch. Starches are used industrially at the higher concentrations, at which they show anomalous viscosity varying with the rate of shear, and, therefore, to predict their industrial performance adequately, tests must be made at these concentrations and the results will depend upon the rate of shear, which, in turn, depends upon the type and dimensions of the viscometer. Thus, only identical instruments will give the same apparent viscosity for a given paste (Morgan and Vaughn, *Ind. Eng. Chem.*, 1943, **35**, 233), and even with these the method by which the paste is prepared must be accurately standardised. When starch is heated with water above a definite temperature, the paste viscosity increases to a maximum value as the granules swell and then, with further heating, decreases continuously. These changes occur at rates that depend upon the variety of starch, its method of manufacture and the technique used in the preparation of the paste, especially the rate of heating, the stirring rate, the dimensions of the stirrer and the kind of stirring motion used. Since starch pastes must be cooked at temperatures near 100° C., loss of water by evaporation will alter the result as will differences in hydrogen ion activity and the presence of many organic and inorganic substances, including the ions present in hard water (Richardson and Waite, *J. Textile Inst.*, 1933, **24**, T383).

In present methods for the industrial testing of starch only a single determination of paste viscosity is usually made, but this determination, even when made by a carefully standardised procedure, is inadequate to characterise a starch completely. Instruments designed either to permit a series of determinations to be made on the same paste or to produce a continuous record of the changing viscosity have been used for research but not for general testing in the industry. The Corn Industries viscometer now to be described, has been developed for the industrial testing of starch products.



The construction of the viscometer is shown in the diagram. The stirring device consists of two parts. A synchronous electric motor (not shown) drives the outer part of the scraper (19) clockwise at 24 r.p.m. through a worm (8) and worm gear (9). The shaft of the scraper is hollow and concentric with it is the shaft of the propeller (20). The propeller is driven counterclockwise at 60 r.p.m. by means of the synchronous motor, but the power is transmitted to it through a planetary differential gearing consisting of spur gears (4), (5), (6), and (7). The torque to which the propeller is subjected as it turns in a viscous medium is likewise transmitted through the differential gearing to drum (3), which is attached by a cable (2) to a dynamometer (not shown) built into the recorder (1).

A strip recorder chart drive is used and the pen arm is actuated by the torque on the propeller. The torque is balanced by a dynamometer consisting of a weight arm that moves through an arc. By means of easily interchangeable weights the full scale of the chart can indicate 250, 500, 1000, or 2000 g.-cm., and weights may be changed even during a test without altering the standardisation of the dynamometer. Such a system is preferable to one using torsion wires, where considerable time is required to change a wire and where the change necessitates recalibration of the instrument. By means of a counterpoise in the dynamometer the internal friction of the mechanical parts is balanced and the weight of the counterpoise is adjusted so that the reading for water is zero.

In operation, as the shaft which drives the scraper (19) is set in motion, the arm upon which the planetary gears (5) and (6) are mounted revolves about this shaft. Since gear (4) does not freely rotate, because it is connected with the dynamometer, it causes gears (5) and (6) to rotate on their axis as they revolve about the scraper shaft. The rotation of the gear (6) drives the propeller counterclockwise through gear (7).

It is possible to uncouple the stirring device at (11) and the head can then be swung to one side to allow removal of the stirrer and starch beaker (17). Since the inner shaft to propeller (20) rotates freely, two spring pins at (10) provide a means for holding it during the coupling operation.

The scraper blades at the side are hinged in such a manner, that, as the stirrer turns, the pressure against the blades causes them to swing outward. Similarly the lower blade rests lightly on the bottom of the beaker. This prevents the formation of a thick adhering layer on the walls of the beaker, and the propeller, in addition to providing a means of measuring the torque, maintains the uniformity of the paste.

The well-insulated water-bath is heated by a 1000-watt immersion heater (18) and is controlled to within 0·2° C. by an adjustable thermostat with a range of 10° to 150° C. Thermometers are placed in the paste and in the bath. The stirrer and the 1-litre cooking beaker are of stainless steel, and the beaker has a condenser cover in two sections which minimises loss of water and which is fitted with connections for cooling water if that is desired. The loss of water at 94° C. in 25 min.

0·14 per cent. and in 55 min. 0·21 per cent., whereas the loss in some commercial recording viscometers may reach 5 per cent. A satisfactory temperature range is 92° to 96° C.; the paste reaches its maximum temperature in 15 min. and sufficient information for routine testing is obtained in 20 min.

To make a determination the metal beaker is placed in the water-bath and the stirring device is attached and started. The starch required for 1000 g. of paste is stirred to a slurry free from lumps in the entire quantity of water needed, at room temperature. This is poured rapidly into the beaker and the recorder is started. The condenser cover is then fitted and the test proceeds automatically.

The precision of the instrument is within 1 per cent. with the same operator and within 2 per cent. with different operators in different laboratories. The permissive tolerance in weighing the starch is 0·05 g. and in measuring the water 1 ml. The volume of the paste may be varied by as much as 100 ml. without affecting the readings.

To calibrate the instrument the viscosities in absolute units of a number of corn syrups, covering the entire range of the viscometer were determined by means of an Ostwald - Cannon - Fenske viscometer which, in turn, had been calibrated with oils of standard viscosity. The data indicated an approximately linear relationship between the measured torque and the viscosity in poises. With the instrument described viscosity can be measured up to about 55 poises; this covers the range for 3 per cent. unmodified tapioca or potato starch or 4 per cent. unmodified maize starch to 6·5 per cent. tapioca or 9 or 10 per cent. maize starch. With materials that do not require cooking, the outer part of the agitator can be removed and the propeller can be replaced by a disc.

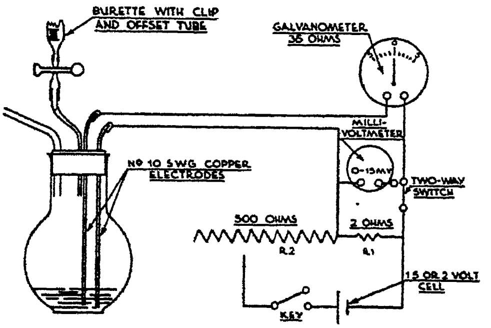
The charts have a scale from 0 to 100, time intervals (1 min.) being shown by arc graduations. The recorded viscosity curve shows viscosity values at all times during the test.

Points on the curve of special significance are the point at which the granules have swollen sufficiently to cause a noticeable increase in viscosity, the point of maximum viscosity, and the decrease of viscosity (breakdown) following the maximum point. Interpretation of the curve provides information about differences existing between production batches of the same product and distinctions between products of different types of manufacture and from different sources. A. O. J.

Polarographic Study of Alkaloids. Part V. H. F. W. Kirkpatrick (*Quart. J. Pharm.*, 1946, **19**, 526–535)—The origin of the polarographic waves previously reported in this series (*cf. Idem, ibid.*, 1946, **19**, 127) has been traced to the halide present when the hydrochlorides or hydrobromides of the alkaloids were used. These waves were produced in very dilute solutions and appear to be catalytic in nature. More concentrated solutions of a number of alkaloids produce characteristic waves, which also appear to be catalytic, and are due to the alkaloids. Atropine, hyoscyamine, hyoscine, homatropine, cocaine, nicotine, sparteine, and pilocarpine give rise to characteristic waves at concentrations of the order of 10^{-3} *M*. Colchicine produces a well-defined reduction wave in the same range of concentration. Aconitine and ephedrine exhibit no polarographic reactions.

J. G. W.

Electrometric Method for the Estimation of Sucrose and Reducing Sugars. S. E. Coalstad (*J. Soc. Chem. Ind.*, 1946, **65**, 230–232)—The dead-stop titration method of Foulk and Bawden (*J. Amer. Chem. Soc.*, 1926, **48**, 2044) has been adapted to the volumetric estimation of reducing sugars with Fehling's solution without preliminary clarification of the sugar solutions. The apparatus (see Fig.) consists of a 250-ml. fat extraction flask fitted with a rubber stopper carrying a burette, a steam vent tube, and two electrodes of clean copper wire of No. 10 S.W.G. The electrodes dip into 10 ml. of Fehling's solution, and are connected through a galvanometer to a shunted battery so that an e.m.f. of 10 millivolts is impressed on them. A suitable galvanometer is the combination mirror and pointer instrument made by the Cambridge Instrument Co. and having a resistance of 35 ohms. When pure invert sugar solution is titrated into Fehling's solution in this apparatus, the galvanometer is fully deflected to one side during the titration and swings almost to zero at the end-point. In a series of ten titrations of pure sucrose inverted with sulphuric acid the mean was 25·62 ml., the theoretical being 25·64 ml.



Irregularities in the titration of impure sugar solutions, such as molasses, raw sugar, and juice were overcome by adding 0·1 to 0·4 g. of purified animal charcoal to the reaction mixture; the charcoal was purified by boiling 1 kg. of commercial bone-char with 6 litres of diluted hydrochloric acid (1 + 4) for 30 min., washing under suction on a Buchner funnel with hot water until free from chlorides, drying at 100° C., and pulverising in an agate mortar. It is sometimes an advantage to use a mixture of purified animal and vegetable carbons together (Paine and Badollet, *Facts about Sugar*, 1927, **22**, No. 3).

In titrations of unclarified mixed sugar-mill juice (22·3° Brix, diluted four-fold) against 10 ml. of Fehling's solution, with 0·1 g. of purified charcoal in the reaction mixture, the results agreed very closely with those obtained by the methylene blue method after clarification with neutral lead acetate and de-leading with phosphate - oxalate mixture. With unclarified solutions of cane molasses, satisfactory titrations were obtained by the electrometric method, before and after inversion.

For the determination of reducing sugars in raw cane sugars, using 100 g. of sample per 200 ml. of solution, larger amounts of purified charcoal were necessary (0·3 to 0·4 g.); percentages of reducers of the order of 0·14 were thus determined.

E. M. P.

Inorganic

Explosion in the Determination of Cobalt as Potassium Cobaltinitrite. D. B. Broughton, M. E. Laing, and R. L. Wentworth (*Anal. Chem.*, 1947, **19**, 72)—A solution of potassium nitrite was added to a solution of cobaltous nitrate buffered with sodium acetate and acetic acid. After being allowed to stand warm, overnight, the precipitate of potassium cobaltinitrite was filtered off. The filtrate was heated over a small flame to coagulate the small amount of colloidal potassium cobaltinitrite that had passed the filter. When evaporation had reduced the volume of the suspension by about one half, the liquid turned purple and exploded violently. Repetition of the experiment invariably produced the same effect, even when the amount of cobalt in the filtrate was very small. In the absence of cobalt, mixtures of sodium acetate, acetic acid, and sodium nitrite do not explode. It is advisable to avoid prolonged evaporation of the above filtrate and to discard it if a blue or purple coloration appears.

B. A.

Separation of Copper and Zinc with Sodium Thiosulphate in the Analysis of Brass. I. Sarudi (v. Stetina) (*Z. anal. Chem.*, 1938, **115**, 260–265; *cf.* Vortmann, *Ibid.*, 1881, **20**, 1881)—The use of thiosulphate to separate zinc and copper has the disadvantage that, in the subsequent treatment of the filtrate from the cuprous sulphide, finely divided sulphur is formed. This may be rendered easily filterable by evaporating the filtrate, acidified with sulphuric acid, almost to dryness, "fuming" the residue, and adding water, whereupon flocculent sulphur separates out.

Method—In analysing brass, tin is first removed as metastannic acid, then lead as lead sulphate, before an aliquot part of the solution corresponding to 0·4 g. of the sample is taken. Dilute the aliquot to 100 ml., and just neutralise with aqueous ammonia and dissolve the slight precipitate in one or two drops of dilute sulphuric acid. Add 50 ml. of 10 per cent. sodium thiosulphate solution, acidify with 20 ml. of diluted sulphuric acid (1 + 5), and heat to boiling with constant stirring. Boil until the supernatant liquid is water-clear and filter. Determine the copper by igniting the sulphide to oxide in a silica crucible, fusing with 3 g. of potassium pyrosulphate, dissolving the melt in water, and, after warming with 1 to 2 ml. of 2 *N* acetic acid and cooling, adding 5 g. of potassium iodide, and titrating with standard sodium thiosulphate solution.

Evaporate the filtrate from the copper precipitation to fuming and leave it to cool. Add 10 ml. of water, rinse the cover-glass, and stir. The bulk of the sulphur is flocculent, but traces of colloidal sulphur remaining can be oxidised by adding 8 to 10 ml. of saturated bromine water. Boil off the excess of bromine, filter off the sulphur, and wash with hot water. Iron, nickel, and zinc may then be determined by the usual methods.

Results—On solutions containing only copper and zinc; the copper values obtained show a maximum deviation of 1 part in 330, on 8 samples containing from 0·13 to 0·25 g. of copper. The zinc contents varied from 0·11 to 0·16 g. and showed a maximum deviation of 1 part in 400. In three analyses of a brass, agreement between the values for the copper content (61·4 per cent.) was within 1 in 877, and for the zinc (37·8 per cent.), within 1 in 756.

M. E. D.

Determination of Chromium by Oxidation with Perchloric Acid. S. Schuldiner and F. B. Clardy (*Ind. Eng. Chem.*, *Anal. Ed.*, 1946, **18**, 728–729)—The method avoids loss of chromium by volatilisation as chromyl chloride, reduction of chromium by the hydrogen peroxide formed in the reaction flask, and incomplete oxidation due to inadequate heating. The apparatus consists of a 500-ml. boiling flask connected to an air condenser through an inverted U-tube; the joints *must* be of ground glass. Any chromium volatilised is collected in water, which hydrolyses the chromyl chloride to chromic acid for volumetric determination.

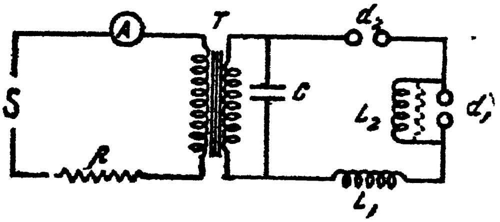
Procedure—Place 0·2 g. of the sample of high-chromium steel in the flask and dissolve it in 10 ml. of hydrochloric acid and 5 ml. of diluted nitric acid (1 + 1) by warming. Add 25 ml. of perchloric acid and heat to incipient fuming. Then connect the air condenser, which should dip 0·25 in. below the surface of 75 ml. of water in a 150-ml. beaker. Heat the flask until the fumes clear and the perchloric acid refluxes down the walls of the flask. Turn off the electric heater and 2 or 3 min. later disconnect the condenser from the ∩-tube and flask; then cool the flask rapidly, first in a current of air and then with tap water, to avoid reduction of dichromate by hydrogen peroxide formed in the reaction flask. Rinse the inside of the connecting tube, collecting the washings in the flask, and rinse the condenser similarly into the beaker. Combine the two solutions in the reaction flask and heat until all the chlorine is driven off; the volume of liquid should be about 250 ml. Cool the solution, and titrate with standard ferrous ammonium sulphate, ferrous *o*-phenanthroline being used as indicator.

Back-suction is avoided by using an air condenser 2 ft. long and of internal diameter 1 in., and by protecting the flask from draughts. The condenser must be connected before the solution turns from green to orange. In presence of vanadium, add excess ferrous ammonium sulphate and titrate back with permanganate, without adding any indicator.

Results—Of six values given, five are within 1 part in 900 of the chromium present, although the evolved chromium is as high as 2·4 per cent. of the total, without deliberate overheating. Complete recovery is proved by treating samples containing contents of from 0·27 to 18·7 per cent. of chromium. The method avoids the use of an empirical factor and chromium can be calculated stoicheiometrically. M. E. D.

Spectro-analytical Determination of Boron in Steel. N. M. Gegetschkori and O. B. Falkova (*Zavod. Lab.*, 1945, **11**, 71–74)—Two distinct methods are described dependent upon the content range of boron (0·1 to 1 per cent. and 0·001 to 0·1 per cent.). A spectrograph having a dispersion of not less than 8 A. per mm. at a wavelength of 2500 A. is required so that the boron line 2496·7 A. may be separated from the iron line 2496·54 A.

Method for boron within the range 0·1 *to* 1 *per cent. Preparation of synthetic standards*—Dissolve 3 g. of carbon steel in 100 ml. of diluted hydrochloric acid (1 + 1) (solution is not complete), add a small amount of nitric acid, heat to effect solution, filter, and to portions of the filtrate add boric acid to give solutions equivalent to boron contents of 0·1, 0·2, 0·5, and 0·83 per cent. These solutions are to be used for finding the boron contents of samples, carefully selected for homogeneity, that will then be used as solid standards. *Standardisation*—Dissolve 3 g. of each of the selected samples in hydrochloric acid with the addition of nitric acid, as above, but in this case under a reflux to avoid loss of boron. With each solution, either the synthetic standard or the sample to be used as standard, place one drop, using a micro-pipette, on the flat surface of a graphite electrode, previously sparked for 3 min. to render it porous, and then dry it for 7 to 8 min. *in vacuo.* Then, using as the second electrode a graphite rod with hemispherical end, photograph the spectrum of each of the solutions twice on the same plate, and measure photometrically the intensities of the boron line 2496·7 A. and the iron line 2510·8 A. The graph of Δ S (difference of blackening) against the logarithm of the concentration for the standard solutions gives the content of boron in the sample-standards.



Method—The excitation circuit (see Fig.) is a modified Raiski spark (*J. Tech. Physics, Russ.*, 1939, **9**, 1719; *cf. Metal Industry*, 1947, **70**, 23). The analytical spark gap is between the solid sample, on the surface of which is machined a clean surface of area 1 sq. cm., and a rod of Armco iron with a hemispherical end. Circuit details are: transformer 120/13,000 v.; condenser 0·01 μF.; coil L_1 26 turns, diameter 10 cm., length 20 cm.; coil L_2 self-inductance 85,000 cm.; d_1 analytical spark gap; d_2 spark gap between two tungsten discs diameter 16 mm., 2·2 mm. apart, mounted on iron rods; and current strength 3·5 amp. Spectra of the sample to be tested and three standards are taken on the same plate. The accuracy attainable is about 3 to 5 parts in 100, using the lines 2496·7 A. (boron) and 2510·8 A. (iron).

Method for boron within the range 0·001 *to* 0·1 *per cent.*—Because of background, the above-described method cannot be used for contents of boron less than 0·05 per cent. With the activated A.C. arc instead of the condensed spark, contents as low as 0·01 per cent. may be determined though with difficulty, when solid samples are used. The use of a powdered sample, however, greatly improves the sensitivity and, in addition, reduces the effect of possible heterogeneity of material and facilitates the preparation of standards. *Preparation of standards*—Obtain filings or sawings from a sample of high boron content, say 0·09 per cent., as determined by the first method, and pass them through a sieve with apertures 0·3 mm. in diameter. Treat a sample of Armco iron similarly, and take proportions of the two powders that, on mixing, will give the desired boron contents. Mix the powders (total weight about 2 g.) for 15 to 20 min. and use about 200 mg. for the test.

Method—File the sample under test in several places to obtain a total weight of filings of about 1 g. and sieve them. Place a portion in a small hole, 7 to 8 mm. in diameter and 2·5 to 3 mm. deep, that has been drilled in a small aluminium block. This serves as the lower electrode. For the upper, use an Armco iron rod with hemispherical tip. With an arc gap of 2·5 mm., a current of 5 amp., and an exposure of 30 sec. photograph two spectra of each sample and two of each standard, using fresh portions of the filings each time, and exciting the spectra by means of an activated A.C. arc circuit (*cf. Metal Industry*, 1945, **67**, 226). Measure on the microphotometer the lines boron 2496·7 A., and iron 2495·5 A., and obtain the boron contents of the samples by means of a calibration graph. The method is sensitive to 0·001 per cent. of boron; the experimental error is about 15 parts per 100. G. S. S.

Determination of Phosphorus in Austenitic Chromium-Nickel Steels. Methods of Analysis Committee of the Metallurgy Division of B.I.S.R.A. (*J. Iron and Steel Inst.*, 1947, **155**, 373–391)—The complete recovery of phosphorus by the phosphomolybdate method is dependent on the following factors. The presence of chromium is advantageous, as is also intense fuming with perchloric acid, or strong baking at 350° C. in non-perchloric acid methods. In absence of chromium, decomposition by *aqua regia* prior to fuming with perchloric acid is effective. Deviations between 30 and 50 per cent. of the total phosphorus content are incurred by inadequate fuming or baking. Co-precipitation of arsenic is prevented by removing it as its volatile bromide; ammonium bromide is used in *aqua regia* solutions, but hydrobromic acid is necessary in perchloric acid treatments. Co-precipitation of molybdic acid is avoided by precipitating at 80° C. rather than at 100° C. Molybdate reagent is more easily prepared from solid ammonium molybdate than from molybdic oxide, and is more stable, although results by the two methods are similar.

Preparation of reagent—(*a*) To 1 litre of nitric acid (sp.gr. 1·20) containing 240 g. of ammonium nitrate and 0·04 g. of microcosmic salt, add, with repeated shaking, 1 litre of 15 per cent. ammonium molybdate solution. Set aside overnight, and filter immediately before use. (*b*) Suspend 100 g. of pure molybdic oxide in 240 ml. of water, and add 170 ml. of aqueous ammonia (sp.gr. 0·880). Pour the solution, with repeated shaking, into 1250 ml. of nitric acid (sp.gr. 1·20) containing 0·04 g. of microcosmic salt. Set aside overnight, and filter immediately before use.

Procedure for straight chromium - nickel steels—Weigh 2 g. of the sample into a 400-ml. conical beaker, and dissolve it in 15 ml. of nitric acid (sp.gr. 1·42) and 25 ml. of hydrochloric acid (sp.gr. 1·16) by digestion. Add 20 ml. of perchloric acid (sp.gr. 1·54), heat to fuming, and continue fuming strongly in the covered beaker for 10 min. after the chromium is apparently fully oxidised. Cool slightly, add 10 ml. of hydrochloric acid, and warm gently to obtain a clear solution, then add 30 ml. of diluted hydrobromic acid solution (1 + 2) and concentrate the solution until the chromium begins to re-oxidise. Cool slightly and re-dissolve in 25 ml. of water by warming. Filter, and wash the residue with hot water. Collect the filtrate in a 400-ml. conical beaker and dilute to 75 ml., then add 8 g. of ammonium nitrate and heat to 80° C. Add 35 ml. of cold molybdate reagent, shake the solution until the precipitate forms, and allow to stand for 30 min., or with low phosphorus contents (< 0·02 per cent.) stir vigorously for 4 min. and allow to stand for 1 hr. Complete the determination according to the First Report (*J. Iron and Steel Inst.*, 1942, 279–293P).

Determine the blank correction by running a parallel treatment on a steel of known phosphorus content; or, take a large quantity of the reagents, add 0·1 g. of a pure ferric salt, and precipitate the ferric phosphate - hydroxide. Re-dissolve it in acid and recover the phosphorus as phosphomolybdate under normal precipitation conditions. The blank is of the order of 0·001 to 0·002 per cent. of phosphorus.

Steels containing tungsten, titanium, cobalt, zirconium, vanadium, tin, and arsenic—With solutions obtained from steel and added salts of these metals, it has been shown that titanium and vanadium inhibit precipitation of the phosphomolybdate complex, while vanadium discolours the precipitate owing to some co-precipitation. Niobium and tantalum tend to produce low results as some phosphorus is removed in the earth-acid precipitate, from which it may be recovered. Zirconium also gives low results as insoluble zirconium phosphate forms and is removed with the insoluble residue. Phosphorus is also occluded on tungstic acid, and is removed in the insoluble residue; the loss of phosphorus is proportional to the tungsten content.

The effects due to the presence of these elements can be avoided by various means, but the devices having the most general application are incorporated in the recommended method.

Procedure—Treat the sample as for straight chromium - nickel steels until a clear solution is obtained prior to the hydrobromic acid treatment (Note 1). Boil this solution for 5 min., and filter through paper into a 400-ml. conical beaker, washing the residue with hot, diluted hydrochloric acid (1 + 49). Reserve the filtrate if the residue is to be treated (Notes 2, 3, and 4), and treat the residue appropriately. Combine the filtrate with the acid extract of the residue (Notes 2 and 3), add 10 ml. of hydrobromic acid (sp.gr. 1·46), and evaporate to fuming, avoiding re-oxidation of the chromium. Cool slightly, re-dissolve in 25 ml. of hot water, and dilute to 75 ml. (Note 5). Add 8 g. of ammonium nitrate, heat to 80° C., and precipitate the phosphorus with 35 ml. of cold molybdate reagent. Complete the determination, and blank, as with straight steels.

Notes—1. To ensure the separation of the acid oxides of tungsten, niobium, and tantalum, re-evaporate to fuming after reduction of the hexavalent chromium, cool, and extract with 25 ml. of hot water. Boil vigorously for 1 min., simmer for 5 min., and allow the residue to settle before filtering as described.

2. To recover co-precipitated phosphorus in the presence of niobium, tantalum, zirconium, or tungsten, transfer the residue and paper to a platinum crucible and ignite at dull red heat. Cool, moisten the residue with a few drops of water, add 2 ml. of hydrofluoric acid and 5 drops of nitric acid (sp.gr. 1·42), and evaporate to dryness. Fuse the residue with 3 g. of potassium bisulphate, extract with 25 ml. of 15 per cent. tartaric acid solution, and transfer to a 200-ml. conical flask. Add 4 ml. of "arsenic solution" (0·25 g. of arsenious oxide dissolved in 10 ml. of nitric acid, concentrated to a small bulk, dissolved in a further 10 ml. of nitric acid, and re-evaporated to 5 ml., then diluted to 100 ml.), 5 ml. of "magnesia" solution (15 g. of magnesium chloride and 15 g. of ammonium chloride dissolved in water, and diluted to 100 ml.), and dilute to 50 ml. Neutralise to bromocresol purple indicator with concentrated aqueous ammonia, and add 5 ml. in excess, then cool to 15° C., shake vigorously for 5 min., and allow to stand for 1 hr. Filter the precipitate on to a tightly-packed paper pad, and wash 5 times with small quantities of cold, 3 per cent. aqueous ammonia solution. Dissolve the residue from the pad with 2 quantities of 10 ml. of diluted hydrochloric acid (1 + 1), and add the solution to the filtrate from the initial insoluble residue. Wash the pad further with hot water 6 times. Proceed with the hydrobromic acid treatment as described.

3. When zirconium only of these other acid-forming elements is present, remove the silica as described in Note 2, fuse the residue with 0·5 g. of sodium carbonate, and extract the melt with hot water. Filter, and wash the residue with hot water. Acidify the filtrate with diluted hydrochloric acid (1 + 1), and add to the filtrate from the original insoluble residue.

4. Samples containing up to 2 per cent. of titanium and/or vanadium, but without columbium, tantalum, zirconium, and tungsten, need not be treated for co-precipitated phosphorus.

5. In the presence of titanium, vanadium, and zirconium, a high nitric acid concentration is necessary to inhibit the retarding effect of the first two on the phosphomolybdate precipitation, and to prevent precipitation of insoluble zirconium phosphate.

To the solution, which must not exceed 65 ml. in volume, add 35 ml. of nitric acid (sp.gr. 1·42), and dilute to 100 ml. with water. Add 8 g. of ammonium nitrate, heat to boiling, and boil for 2 min. Cool to 80° C., add 35 ml. of cold molybdate reagent, and proceed as described. With this high acid concentration, vigorous agitation with a glass rod during precipitation is essential. M. E. D.

Use of Pyrophosphoric Acid in the Determination of Nickel in Cast Iron and Steel. R. T. Kononiuk (*Zavod. Lab.*, 1945, **11**, 103)—Pyrophosphoric acid is superior to citric acid for forming a complex compound with iron in the volumetric cyanide determination of nickel in iron and steel since the end-point is more easily found in the light yellow solution; citric acid gives a darker colour. It is also recommended on grounds of economy, although it is stated that the pyrophosphoric acid must be freshly prepared by boiling A.R. phosphoric acid in a bath at a temperature not less than 300° C. until bubbles cease to form (about 3 hr.); the pyrophosphoric acid should be used within 12 hr.

Procedure—Dissolve 1 g. of sample in 30 ml. of mixed acid (95 parts of diluted hydrochloric acid (1 + 1) and 5 parts of concentrated nitric acid), cool the solution, mix with 12 ml. of freshly prepared pyrophosphoric acid, and add 200 ml. of distilled water. Neutralise with aqueous ammonia, dissolve any precipitate by addition of an excess of aqueous ammonia, cool again, add 2 to 5 ml. of silver nitrate solution, clear the solution, if necessary, by addition of a further quantity of ammonia, add 5 ml. of potassium iodide solution, and titrate with potassium cyanide solution until the opalescence due to silver iodide completely disappears.

G. S. S.

Use of Phenylanthranilic Acid in the Volumetric Determination of Molybdenum in Steel and Ferro-molybdenum. E. I. Fogelson and N. V. Kalmikova (*Zavod. Lab.*, 1945, **11**, 31–34)—Reduction of molybdate to quinquevalent molybdenum by means of mercury is superior in routine analysis to reduction to tervalent molybdenum by means of zinc amalgam, since the reduced solution is not oxidised by the air, and vanadium, which is reduced to the tervalent state, does not interfere when the solution is titrated, say with sodium vanadate. In addition, evaporation with sulphuric acid to complete removal of nitric acid is unnecessary and the reduction may be carried out in hydrochloric acid solution (3 *N*).

Determination of molybdenum in alloy steel—Dissolve 1 to 2 g. of steel in 40 ml. of diluted hydrochloric acid (3 + 1), add nitric acid dropwise until the vigorous reaction ceases and then 2 ml.

more, and boil until any tungstic acid present becomes yellow. Evaporate to dryness after washing down the cover and the sides of the beaker, cool, add 50 ml. of diluted hydrochloric acid (1 + 4), heat to dissolve the salts completely, filter, and wash the insoluble material with hot, diluted hydrochloric acid (1 + 10). Add to the filtrate 20 per cent. sodium hydroxide solution until the solution is weakly acid, heat to boiling, and run the solution in small portions, with stirring, into 160 ml. of hot, 10 per cent. sodium hydroxide solution in a 500-ml. graduated flask, cool, make up to the mark, allow the precipitated hydroxides to settle, and filter through a dry paper, rejecting the first portion. Pipette 50 ml. of the filtrate into a conical flask, add 25 ml. of concentrated hydrochloric acid, cool to room temperature, and transfer to a Someya, or similar, reduction apparatus containing 12 to 15 ml. of mercury, depending on the volume of the lower tube. Close the apparatus and shake with the mercury for 5 min. Pour the solution through a filter into a 250-ml. conical flask, wash with water, add 14 ml. of concentrated sulphuric acid to the filtrate, cool to room temperature, add seven drops of a solution of phenylanthranilic acid (0·1 g. with 0·1 g. of sodium carbonate in 50 ml. of water), and titrate with 0·03 *N* sodium vanadate solution (prepared by dissolving 2·87 g. of vanadium pentoxide and 3 g. of sodium hydroxide by heating in 100 ml. of water, neutralising to litmus with diluted sulphuric acid (1 + 1) and adding 760 ml. of water and 140 ml. of concentrated sulphuric acid). After filtration, establish the titre by titration with ferrous sulphate or against a standard steel put through the same process as the sample; 1 ml. is equivalent to approximately 2·88 mg. of molybdenum. Use a micro-burette and titrate until a permanent reddish-violet colour appears.

Determination in ferro-molybdenum—Dissolve 0·5 g. of the finely ground, sieved sample in 15 ml. of diluted nitric acid (1 + 2), then add 5 ml. of concentrated sulphuric acid, and evaporate to fuming. Cool, add 15 to 20 ml. of water, heat to dissolve the salts, filter if necessary, neutralise with 20 per cent. sodium hydroxide solution, boil, pour into 100 ml. of 10 per cent. sodium hydroxide solution in a 500-ml. graduated flask, heat to boiling, cool, make up to the mark, leave to settle, pipette off 50 ml. of the filtered solution and proceed as above.

G. S. S.

Two New Selective Cerium Reactions. E. Herzfeld (*Z. anal. Chem.*, 1938, **115**, 421–423)—Solutions containing a range of from 1 to 250 μg. of cerium and of related earths were prepared by dilution of aqueous solutions with 50 per cent. glycerol. On adding 1 drop of concentrated aqueous ammonia, 1 drop of 1 per cent. benzidine in 50 per cent. glycerol in ethanol, and 3 drops of glacial acetic acid, a green coloration appears only in solutions containing more than 62 μg. of cerium. There is no Tyndall effect. No coloration is formed by the other rare earths.

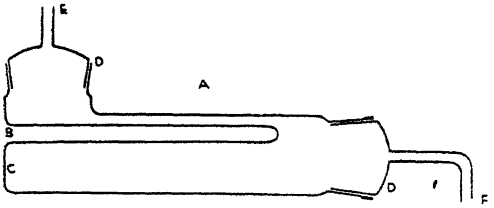
Addition of 5 drops of 1 per cent. tannin in 50 per cent. glycerol, and 5 drops of saturated sodium acetate in the same solvent to the solutions prepared as above gives, after one hour, a blue-violet coloration for cerium in a Tyndall-meter. Other earths, except europium, give grey colorations; the europium colour is yellow. The test is sensitive to 8 μg. of cerium.

Addition of 3 drops of the sodium acetate in glycerol solution, and 2 drops of carminic acid solution, prepared by diluting tenfold with water a saturated solution of the acid in ethanol, gives a red coloration with all earths after standing for 24 hr. In a Tyndall-meter by quartz light the colour is blue-violet with a reddish cone. The sensitivity is the same as in the tannin reaction. In the cerium series only does the intensity of the Tyndall cone decrease with increasing cerium content.

In extracting cerium from rare earth preparations, the sensitivity of precipitation with hydrogen peroxide was established as 16 μg. M. E. D.

Separation of the Rare-Earth Elements by Chloride Volatilisation. R. C. Vickery (*J. Soc. Chem. Ind.*, 1946, **65**, 388–390)—A new method of separating the rare earth elements depends upon the different temperatures of volatilisation of the chlorides. For the ten chlorides examined, these temperatures are found to be in a convenient range, 360° to 480° C., when volatilisation is carried out in an atmosphere of chlorine at 1 mm. pressure, *viz.*, $TbCl_3$, 360°; $DyCl_3$, 370°; $GdCl_3$, 395°; $CeCl_3$, 400°; $SmCl_3$, 410°; $ErCl_3$, 420°; $NdCl_3$, 425°; $PrCl_3$, 425°; $YbCl_3$, 425°; $LaCl_3$, 476° C. The following binary mixtures were heated in such an atmosphere for 90 min. at temperatures between the volatilisation temperatures of the two components: lanthanum chloride and neodymium chloride, terbium chloride and cerium chloride, lanthanum chloride and ytterbium chloride, terbium chloride and erbium chloride, and praseodymium chloride and gadolinium chloride. None of the residues contained less than 92 per cent. of the amount of the less volatile chloride taken, or more than a trace of the other chloride. Fractionations of several ternary mixtures by heating at two temperatures gave further satisfactory separations. A natural rare-earth mixture was freed from thorium by extraction of the oxalates with ammonium oxalate solution, and the anhydrous chloride of the mixture was fractionated at 380° C. and 420° C. under a pressure of chlorine of 1 mm. Spectrographic analysis of the three fractions showed that an appreciable separation had been achieved.

The apparatus employed is a silica tube bent to a right angle and flattened at the bend to form an observation window. A narrow tube to carry a thermocouple projects inside the main tube from the flattened end. One end of the combustion tube is connected to a source of chlorine and the other to absorption tubes and a vacuum train. The tube is heated in a resistance furnace.



Procedure—Dehydrate the chlorides by the method of Kleinheksel and Kremers (*J. Amer. Chem. Soc.*, 1928, **50**, 959), insert in a silica boat into the combustion tube at a temperature of 90° C., introduce the chlorine, and raise the temperature slowly to the required value. The volatilised chlorides condense near the observation window.

B. A.

Application of the Polarographic Method of Analysis to Production Control in Non-Ferrous Metallurgy. S. A. Pletenev, T. V. Arefyeva, E. M. Tal, and E. I. Dubovitzkaja (*Zavod. Lab.*, 1946, **12**, 38–58)—New and rapid methods, several of which do not require any chemical separations, for polarographic determinations of metallic impurities are described.

I. Determination of Copper, Lead, and Zinc Impurities in Metallic Cadmium (pp. 39–42)—Although Krössin and Wagner (*Metall. u. Erz.*, 1941, **38**, 199) were unable to separate the lead and cadmium waves in a cadmium chloride base solution, the authors show that lead together with copper (0·005 per cent. and above in each case) may be determined in metallic cadmium to an accuracy of about 1 in 10 without any chemical separations. For the determination of zinc the bulk of the cadmium must be removed, *e.g.*, by metallic aluminium (*cf.* Cozzi, *Annali Chim. Appl.*, 1941, **31**, 227). Results accurate to about 1 in 30 are obtained with contents of zinc from 0·0076 to 0·0170 per cent.

Procedure for copper and lead (time 1·75 hours)—Treat 5 g. of cadmium in a 250-ml. conical flask with 60 ml. of diluted hydrochloric acid (1+3), heat to boiling, add carefully in two portions 2 to 3 ml. of concentrated nitric acid, and, after solution of the metal, evaporate to dryness. Add 10 ml. of concentrated hydrochloric acid, evaporate to small volume, cool, dissolve in 20 ml. of water, transfer to a 50-ml. measuring flask, dilute to the mark with water, and mix. Place 20 ml. of the solution in a polarographic cell, pass hydrogen gas for 20 min., and, using a dropping mercury cathode giving 10 drops per 15 sec., obtain the waves of copper and lead. Calculate the contents of the metals from the wave-heights and from further polarograms taken after addition of known amounts of copper and lead standard solutions.

Procedure for zinc (time 3·5 hours)—Treat 10 g. of cadmium in a 500 to 600-ml. conical flask with 120 ml. of diluted hydrochloric acid (1+3), heat to boiling, and add carefully in two portions 2 to 3 ml. of concentrated nitric acid. Cool, add 20 ml. of diluted hydrochloric acid (1+3), and then 5 g. of aluminium as thin foil or drillings in small portions, introducing the last of these after allowing the solution to stand for 10 to 20 min. This portion should remain clean with no coating of cadmium. Filter off the aluminium and cadmium on cotton wool or paper pulp, wash three times with diluted hydrochloric acid (1+50), using 20 ml. in all, evaporate the filtrate to from 25 to 30 ml., transfer to a 50-ml. measuring flask, make up with water and mix, place 20 ml. in the polarographic cell, pass hydrogen for 20 min., and take the polarogram, the addition method being used for calculating the zinc content.

Maxima suppressors are apparently not required in either method.

II. Control of Solutions and Products in Cobalt Manufacture (pp. 42–46) — Without chemical separations (*e.g.*, by means of dithizone), copper (0·02 to 0·2 per cent.) and lead (about 0·005 per cent.) may be determined directly in a ground solution of cobalt chloride or sulphate containing a maximum suppressor. Nickel (0·1 to 1·2 per cent.) may be determined directly in pyridine solution as shown by Lingane and Kerlinger (*Ind. Eng. Chem., Anal. Ed.*, 1941, **13**, 77), the nickel wave appearing at 0·8 v. and the cobalt at 1·05 v.

Procedure for copper and lead—With cobalt solutions used for obtaining cobalt hydroxide by precipitation, merely add 5 drops of 1 per cent. joiners' glue solution to 20 ml. and polarograph the solution. With dry cobalt hydroxide, dissolve 5 g. in 30 ml. of concentrated hydrochloric acid, evaporate nearly to dryness (dark blue residue), cool, add 15 to 20 ml. of water, and evaporate as before. Dissolve the residue in 15 to 20 ml. of water, transfer to a 50-ml. measuring flask, dilute to the mark, and mix. Take 20 ml., add 5 drops of glue solution, and determine the polarogram. With metallic cobalt, heat gently 5 g. with 30 ml. of diluted nitric acid (3+2), evaporate to from 10 to 15 ml., add 10 to 15 ml. of concentrated hydrochloric acid, evaporate to pastiness, and proceed as with cobalt hydroxide.

Procedure for nickel—With cobalt solutions, pipette 10 ml. into a 50-ml. measuring flask, add 2 ml. of diluted hydrochloric acid (1+1), 2·5 ml. of pyridine, and 2·5 ml. of a 0·2 per cent. gelatin solution, dilute to the mark, mix, and take 20 ml. With cobalt hydroxide, heat 2 g. with 25 ml. of concentrated hydrochloric acid, evaporate to pastiness, add 10 ml. of hydrochloric acid, and evaporate to dryness, avoiding overheating. Dissolve the residue in 25 to 30 ml. of water, dilute to 200 ml. in a measuring flask, and transfer 25 ml. to a 50-ml. measuring flask, add 2·5 ml. each of pyridine and gelatin solution, and proceed as for cobalt solutions. With metallic cobalt, heat gently 2 g. with 25 ml. of diluted nitric acid (3+2), evaporate to 10 to 15 ml., add 15 ml. of concentrated hydrochloric acid, and evaporate nearly to dryness. Repeat the evaporation 2 or 3 times, using 8 to 10 ml. of concentrated hydrochloric acid each time. Add 30 to 50 ml. of water to the residue, dilute to 200 ml. in a measuring flask, and proceed as with cobalt hydroxide.

It is stated that the methods for copper and lead may be applied to nickel solutions, using the nickel salt as the supporting electrolyte in the same way as the cobalt salt.

III. Determination of Copper, Bismuth, Lead, Cadmium, and Zinc Impurities in Metallic Tin, and in Lead-Tin Solders (pp. 46–50)—Here, removal of the main element (tin) appears to be essential. This is effected by 5 or 6 evaporations with concentrated hydrochloric acid, whereby tin is volatilised as stannic chloride. In polarograms of alkaline tartrate solutions (Suchy, *Chem. News*, 1931, **143**, 213), the authors failed to separate the bismuth and copper waves, but in acid solution, provided the *p*H was strictly controlled within the limits 5·2 to 5·6, they succeeded in determining these and the other impurities on single polarograms (*cf.* Lingane, *Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 147, for the separation of bismuth and copper waves at *p*H 5·2). It is recommended that the potential scale be extended for more accurate measurements of wave-heights. Tin may be present in the solution up to 10 times the concentration of the impurity elements.

Procedure for impurities in metallic tin (time 3 hours)—Heat gently 2 g. with 25 ml. of *aqua regia*, add, after solution, 10 ml. of concentrated hydrochloric acid, evaporate nearly to dryness, and repeat the evaporation 4 or 5 times, adding each time 5 to 8 ml. of acid. Finally, evaporate to dryness, avoiding overheating, cool the residue, moisten with 5 drops of hydrochloric acid, and add 10 to 15 ml. of water. Then add 10 ml. of 44 per cent. sodium tartrate solution, 1 ml. of diluted glacial acetic acid (1+2), and 10 drops of 0·2 per cent. methyl red solution. Alternatively, instead of sodium tartrate and acetic acid, add 6 ml. of 40 per cent. tartaric acid solution and 15 ml. of ammonium acetate solution (25 per cent.). Dilute to 50 ml. in a measuring flask, mix, take 20 ml., pass hydrogen gas for 30 min., and obtain the polarogram. Then add standard solutions of the impurity elements to give polarograms for the calculation of contents.

Procedure for impurities in lead-tin solders—Heat gently 2 g. with 40 ml. of concentrated hydrochloric acid and 8 to 10 drops of perhydrol, and evaporate to small volume. Cook, filter from the precipitated lead chloride, washing with diluted hydrochloric acid (1+4), evaporate to dryness, add 8 to 10 ml. of concentrated hydrochloric acid, and again evaporate. Repeat the evaporation with hydrochloric acid several times until tin is completely removed, as shown by the lustrous appearance of the lead chloride (if tin is present, the dry residue is dull and yellow). Moisten with 5 drops of concentrated hydrochloric acid and proceed as with metallic tin.

IV. Determination of Impurities in Metallic Lead (pp. 51–56)—(i) *Antimony* (0·001 to 0·06 per cent.) *and tin* (0·0005 to 0·002 per cent.)—When arsenic is present, several waves due to this element appear in acid solution, necessitating the removal of arsenic before the determination of the other impurities (*cf.* Kriukova, *Zavod. Lab.*, 1940, **9**, 950). Fusion with alkali (Fainberg, *Zavod. Lab.*, 1937, **6**, 36) gives an alkali melt containing the antimony and tin (and arsenic), while any copper and bismuth present remain with the metallic lead. Traces of lead passing into the alkali melt in sufficient quantity to interfere with the determination of antimony and tin are removed by means of sodium sulphide. Arsenic is reduced to metal, and antimony and tin salts to lower valencies, by means of hypophosphite (*cf.* Cozzi, *Annali Chim. Appl.*, 1939, **29**, 442). [*Cf.* B. S. Evans, ANALYST, 1931, **56**, 171.]

Procedure—Fuse 20 to 50 g. of the lead in a polished iron crucible with 2 g. of potassium hydroxide and 3 g. of sodium hydroxide. As soon as the lead melts, stir the contents of the crucible vigorously with a bright iron spatula for 10 min., then stop heating, and rotate the crucible rapidly so as to cause the lead to solidify in the form of a round "crown." Pour the melt into a dry, porcelain crucible and, after cooling, transfer to a 100-ml. beaker, adding 10 to 15 ml. of water. Place 10 ml. of hot, *N* sodium hydroxide in the iron crucible, rinse the spatula, crucible, and "crown" with 5 to 10 ml. of the same solution, remove the "crown," and again rinse the crucible and "crown" with this solution. Collect the washings, which should not exceed 40 to 60 ml. in all, in the same beaker. After solution of the melt, add 3 to 5 ml. of 10 per cent. sodium sulphide solution, heat gently on a water-bath for 5 to 10 min. to coagulate the sulphide precipitate, and filter into a 250-ml. conical flask, washing the insoluble matter 2 to 3 times with small amounts of 1 per cent. sodium sulphide solution. Add 60 ml. of concentrated hydrochloric acid to the filtrate, evaporate to a volume of 50 to 70 ml., filter off the sulphur, washing with hot water, and collect the filtrate in a 100-ml. measuring flask. Add 1 g. of calcium or sodium hypophosphite, boil for 15 min., cool the flask under the tap, add 10 ml. of freshly prepared 1 per cent. gelatin solution, make up to the mark with diluted hydrochloric acid (1+1), and mix. Transfer a suitable aliquot to the polarographic cell, pass hydrogen gas for 15 to 20 min., and obtain the polarogram. Use, for calculation of content, calibration curves obtained on solutions prepared as follows: In three 100-ml. conical flasks place 1 mg., 2 mg., and 5 mg. equivalents of antimony, tin, and arsenic, respectively, in a volume of 30 to 40 ml., add 5 g. of sodium hydroxide followed by 60 ml. of concentrated hydrochloric acid, boil, and evaporate to 50 to 70 ml. Proceed as above. In tests with different quantities of arsenic, no interference with the waves of antimony and tin was observed and the wave-heights were strictly proportional to the contents.

(ii) *Copper* (up to 0·08 per cent.) *and bismuth* (0·005 to 0·013 per cent.)—The determination is similar to that described for metallic tin.

Procedure—Dissolve 5 g. of the lead in 50 ml. of diluted nitric acid (1+4), evaporate to dryness, add 7 to 10 ml. of concentrated hydrochloric acid, again evaporate, and repeat the addition of acid and the evaporation twice. To the cooled residue add 15 ml. of diluted hydrochloric acid (220 ml. of concentrated acid diluted to 1 litre), heat to boiling, cool, filter off lead chloride, washing with the same diluted acid (2 to 3 times with 10 ml.). Evaporate the filtrate nearly to dryness. If oxides of nitrogen appear, evaporate once more with hydrochloric acid. To the cooled, moist residue add 10 ml. of 44 per cent. sodium tartrate solution, 1 ml. of diluted glacial acetic acid (1+2), and 10 drops of 0·2 per cent. methyl red solution (as a maximum suppressor). Dilute to 50 ml. with water in a measuring flask, take a suitable aliquot, pass hydrogen gas for 20 to 30 min., and take polarograms for copper and bismuth, using calibration curves. To obtain the standards, place in 50-ml. measuring flasks 0·25 mg. and 0·5 mg. of bismuth and copper in the form of chlorides, add 10 ml. of 44 per cent. sodium tartrate solution, 1 ml. of diluted acetic acid (1+2), and 10 drops of 0·2 per cent. methyl red solution, dilute to the mark with water, mix, and proceed as above.

(iii) *Zinc* (0·004 to 0·8 per cent.)—Schaikind (*Zavod. Lab.*, 1939, 8, 567) proposed to determine zinc, nickel, cobalt, cadmium, and copper in an ammoniacal ammonium chloride solution after separation of the bulk of lead as sulphate. The authors' comments on this method are that with the small amounts of copper normally present in lead the copper waves in ammoniacal solution are too small for accurate determinations, and the more accurate method for copper given above is recommended, and that when zinc, nickel, and cobalt occur together the waves of these elements become indistinguishable. In absence of cobalt, the zinc and nickel waves are separable. Normally, however, in lead the contents of nickel and cobalt are negligible, and it is sufficient to take the total wave due to zinc, nickel, and cobalt as representing the zinc content. *Procedure*—Dissolve 10 to 20 g. of lead in 100 to 150 ml. (corresponding to the weight taken) of diluted nitric acid (1+4). Boil several minutes to remove oxides of nitrogen, transfer to a 500-ml. measuring flask, precipitate the bulk of lead by adding 5 to 10 ml. of diluted sulphuric acid (1+1), make up to the mark with water, mix, and filter through a dry filter into a dry flask. Place 250 ml. of the filtrate in a porcelain basin, evaporate nearly to dryness, leaving the residue moist, cool, dissolve in 10 to 15 ml. of hot water, and filter off the lead sulphate after a few minutes, washing with cold, 2 per cent. sulphuric acid and, collecting the filtrate in a 50-ml. measuring flask. Add 5 ml. of 4 *N* ammonium chloride, 1 drop of methyl orange solution, and then 25 per cent. aqueous ammonia to neutralise. Add 5 ml. of 2 *N* aqueous ammonia, make up to the mark with water, mix, take 15 to 20 ml., add 10 drops of 1 per cent. glue solution and several small crystals of sodium sulphite, mix, and take a polarogram for the zinc. Calibrate by placing 0·25, 0·5, and 1 mg. of zinc, in the form of chloride, in separate 50-ml. measuring flasks, diluting with water to 20 to 25 ml., and adding aqueous ammonia, etc., as above.

V. Rapid Method of Determining Lead, Cadmium, and Zinc in Ores and Refuse from Ore-enrichment (Zinc, Lead, and Cadmium Ores) (pp. 57–58)—*Procedure for zinc* (0·1 to 0·6 per cent.) *or cadmium* (about 0·15 per cent.)—Boil 1 g. for 20 min. with 20 ml. of diluted hydrochloric acid (2+1), cool the solution, transfer to a 100-ml. measuring flask, add 20 to 25 ml. of water, 5 to 6 drops of 10 per cent. hydrogen peroxide, and 25 per cent. aqueous ammonia solution to precipitate ferric hydroxide and to give an excess of 10 ml. Make up to the mark with water, transfer 25 ml. of the solution to a measuring cylinder, add 10 drops of 1 per cent. glue solution and 0·2–0·3 g. of sodium sulphite, pour part of the solution into a polarographic cell and obtain the zinc and cadmium waves.

Procedure for lead (0·1 to 0·6 per cent.)—Carry out the acid attack as for zinc and cadmium, reduce ferric iron by means of metallic iron, and take a polarogram in the hydrochloric acid solution directly.

The concentration ranges quoted above after the names of the various elements are not limits of applicability but indicate the range of composition of the samples studied.

For polographic determinations of impurities in metallic zinc without chemical separations reference is made to papers by Pletenev and Arefyeva (*Zavod. Lab.*, 1938, 7, 5 and *Trans. All-Union Conf. on Anal. Chem.*, 1943, part II). G. S. S.

Gas Analysis

Colorimetric Method for Determination of Traces of Carbon Dioxide in Air. N. A. Spector and B. F. Dodge (*Anal. Chem.*, 1947, **19**, 55–58)—The carbon dioxide is absorbed by weak standard solutions of sodium hydroxide, coloured with phenolphthalein, and the change in colour measured with a photo-electric colorimeter. The concentrations of carbon dioxide dealt with vary from 0·0005 to 0·0315 per cent. The accuracy of the determination is said to be 10 per cent. on concentrations of 0·001 per cent.

Procedure—Take the cell of a photo-electric colorimeter and adjust the lamp temperature to give a full-scale deflection with distilled water. Make a 0·0001 *N* solution of sodium hydroxide and colour it with phenolphthalein until the light transmission is about 10 per cent. Calibrate this solution as follows with clean "out-of-doors" air, which can be assumed to contain 0·0315 per cent. of carbon dioxide. Fill a calibrated absorption vessel, made like an ordinary, 300-ml., gas sampling tube, with the sodium hydroxide solution after careful purging with a gas free from carbon dioxide such as cylinder-nitrogen made by the liquefaction of air. Run out about 200 ml. to take in a sample of outdoor air and measure the volume. Shake vigorously for a few minutes, run the solution into the carefully purged colorimeter cell, and measure the change in light transmission. In this way, construct a graph in which the "ml. of carbon dioxide absorbed" are plotted as a straight line on a uniform ordinate against the "per cent. of light transmitted" on a logarithmic ordinate. Unknown concentrations of carbon dioxide can then be estimated directly from the graph.

Details concerning the stability of the colour and the application of the method to test air from which carbon dioxide had been partly removed are given.

W. J. G.

Micro-gravimetric Method for the Determination of Carbon Monoxide. J. M. Salsbury, J. W. Cole, and J. H. Yoe (*Anal. Chem.*, 1947, **19**, 66–68)—Air containing 0·002 to 0·1 per cent. of carbon monoxide is passed over Hopcalite at 195° C. and the resulting carbon dioxide weighed. The accuracy of the method is said to be 2 per cent.

Apparatus—The apparatus consists of a purification train comprising calcium chloride, charcoal, soda lime, soda asbestos, calcium chloride, silica gel, and a moisture indicator consisting of cobalt chloride on silica gel. The Hopcalite is in a U-tube and is heated in a bath of boiling decahydronaphthalene at 195° C. Following the Hopcalite are four micro-absorption tubes, like those recommended by Pregl, comprising straight tubes, 190 mm. long, with ground-glass joints sealed with Krönig's cement and connected in the train by means of "aged" rubber tubing. The first tube is packed tightly with magnesium perchlorate trihydrate, the second with magnesium perchlorate trihydrate ($\frac{1}{3}$) and soda asbestos ($\frac{2}{3}$), the third with the first and last fifths of its volume filled with the perchlorate and the middle three-fifths with soda asbestos, while the fourth (control) tube contains equal amounts of these two substances. After the tubes comes a flow meter, a pressure gauge, and means for applying a suction.

Procedure—Condition the Hopcalite by drawing through purified air, then 1 litre of the air to be analysed and then 500 ml. of purified air. Weigh the absorption tubes and connect them into the train. Cool each tube by placing on top a piece of wet flannel. Draw through 500 ml. or 1 litre of the air to be analysed at a rate of 25 to 100 ml. per min., using the flow meter readings and the time to measure the volume of air. Flush the tubes with purified air and reweigh. Calculate the percentage of carbon monoxide from the gain in weight of the second and third tubes less the gain in weight of the control tube.

W. J. G.

Micro-Method for the Determination of Gaseous Olefines. R. Pyke, A. Kahn, and D. J. LeRoy (*Anal. Chem.*, 1947, **19**, 65–66)—Two new methods are described for the determination of olefines in presence of paraffins, the Blacet-Leighton micro-gas analysis apparatus (*cf. Ind. Eng. Chem., Anal. Ed.*, 1931, **3**, 266; and 1933, **5**, 272) being used.

Method—For ethylene-ethane mixtures, make the usual reagent bead on a platinum loop of wire from a paste containing 3 ml. of powdered mercuric acetate and 2 ml. of a 1 per cent. solution of boron trifluoride ethyl etherate (Eastman Kodak Co.) as catalyst in ethylene glycol. For propylene-propane and butylene-butane mixtures, make the bead from 3 ml. of powdered mercuric acetate, 1·5 ml. of water, and approximately 1 g. of mercuric nitrate, and dry the gas with a bead of fused potassium hydroxide. As the mercuric acetate-nitrate reagent is also suitable for ethylene-ethane mixtures, the authors recommend its use in most determinations.

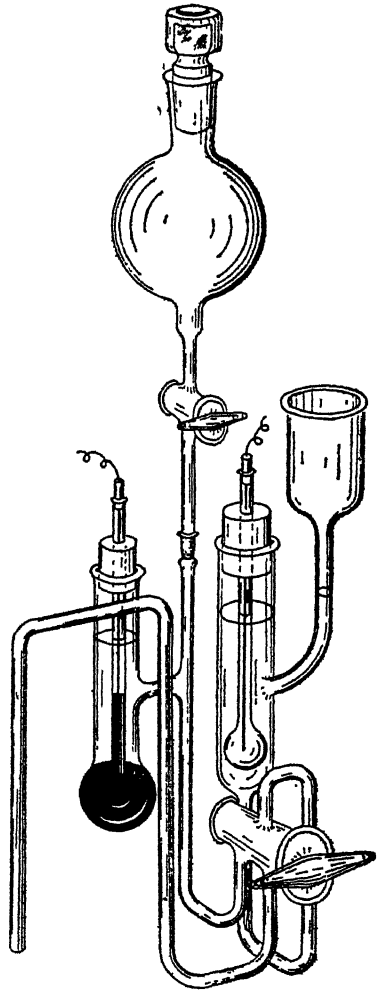
W. J. G.

Physical Methods, Apparatus, etc.

Improved Glass Electrode Cell Assembly. G. E. Coates (*J. Chem. Soc.*, 1945, 489–490)—The cell assembly is for *p*H measurement to ± 0·01 unit, involves a cylindrically symmetrical liquid junction, and can be refilled rapidly without removal from the thermostat. The glass electrode half-cell has a side tube for filling, and may be connected by a three-way tap either to a syphon tube leading over the thermostat side or to the calomel electrode.

The latter is connected by a tap to a reservoir of 3·5 *M* potassium chloride.

Method—Pour 0·05 *M* potassium hydrogen phthalate (*p*H 4·00) into the glass electrode half-cell until the level in the side tube is higher than the top of the syphon. Connect the latter to the cell, flush out and allow to reach the thermostat temperature. Then connect the glass electrode and calomel half-cell and open the reservoir tap until a sharply defined liquid junction has risen slowly about 1 cm. in the limb above the three-way tap. Close the reservoir tap and standardise the *p*H meter. Then connect the glass electrode half-cell with the syphon, flush with water, refill with the solution to be examined, and proceed as before.



J. T. S.

Purification of Carbon Rods for Spectrochemical Analysis. I. Simple Apparatus for Purification by Means of Resistance Heating. P. L. Hoogland. II. Carbon Rods for Spectrochemical Analysis of Solutions. R. Schmidt. III. H. W. Deinum (*Rec. Trav. Chim.*, 1946, **65**, 257–264, 265–269, 270–272)—

I. Spectroscopically pure graphite electrodes were required for an investigation by the cathode layer method of trace elements present in biological material. A modification of the method due to Gatterer (*Spectrochimica Acta*, **1941**, **2**, 49) was adopted for the purification. The starting material used was Siemens-Plania Superbio Negativ Homogen Kohle, 200 mm. long and 7 mm. diameter, having an ash content of about 1 per cent., 60 per cent. of which was iron. The rods were clamped in graphite blocks and a heavy current was passed through them, the best results being obtained by heating the rods for 60 sec. with a maximum current of 650 amp. After treatment, trace impurities of iron, magnesium, calcium, sodium and, occasionally, titanium and copper were present. The boron content was found to be practically unaffected by the treatment and this appeared to form the major residual impurity.

II. The carbon rods used for this method of analysis must be pure, homogeneous, and porous. An attempt was made to use the rods prepared by Hoogland (see I, above); the results obtained, however, were not reproducible. This was mainly due to the "false" porosity of the rods produced by the presence of a central cavity formed during the heating treatment. Nearly all the impregnating solution was distributed down this cavity.

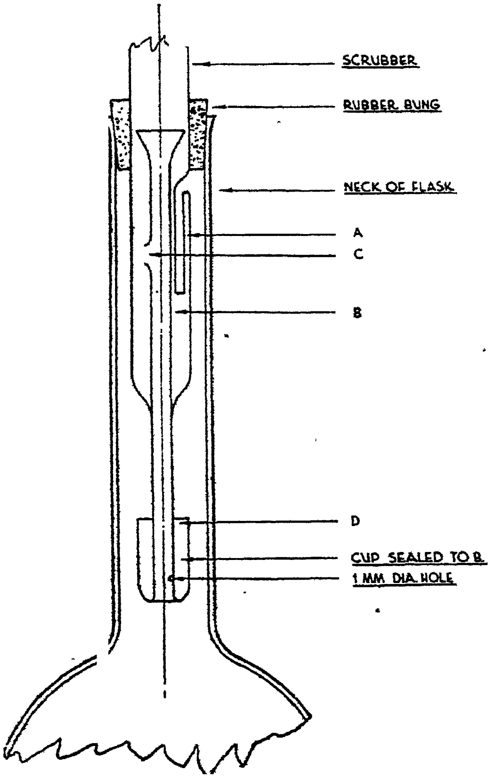
Rods of the same material as used by Hoogland (*loc. cit.*) were of satisfactory purity for use with solution methods when given a less severe heat treatment to a maximum surface temperature of about 3000° C. After the removal of 20 mm. from each end, which gives a rod having fairly constant purity along its length, a final heating at 3000° C. for 2 min. improved the porosity. The rods are, however, not homogeneous in a radial direction and, for the analysis of solutions, the very porous outer layer should be removed, and the electrodes prepared from the rods pre-heated before impregnation by passing an electric discharge between them.

X-ray diffraction studies show that the conversion of the nearly amorphous carbon into graphite by the heat-treatment is not complete.

III. The maximum temperature (*cf.* Hoogland, see I, above, and Schmidt, see II, above) to which a carbon conductor can be heated by direct passage of current has been measured. Temperatures exceeding 3400° K. could be attained, but at these high values, the rod tended to explode owing to the development of a relatively high vapour pressure within it. The surface temperature observed at various electrical loadings was in fairly good agreement with the calculated values.

D. A. P.

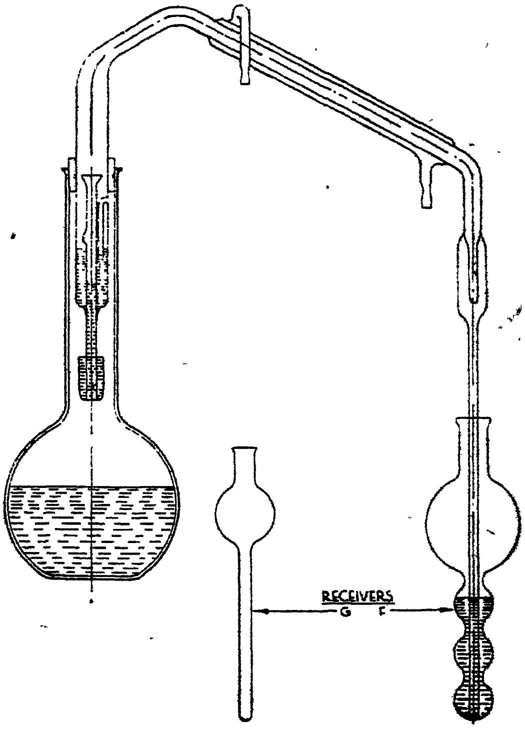
Improved "Kjeldahl Trap" and Ammonia Distillation Apparatus. A. Lovecy (*J. Soc. Chem. Ind.*, 1946, 65, 224–226)—The main object of the apparatus illustrated is to prevent the carrying-over of non-volatile alkali during the quantitative distillation of ammonia from alkaline solution. The vapour stream is scrubbed in passing through water which is practically at boiling-point. The body of the apparatus is approximately 20 mm. in outside diameter. The cup sealed to B is so placed that vapour from the flask cannot escape by this route owing to the hydrostatic pressure provided by the difference in levels between C and D. Vapour therefore escapes through the dip-tube A and is thus brought into contact with the scrubber-liquid. The vigorous frothing occurring at this point is reduced by the conical tap of the central tube, which breaks the films of foam.



Test experiments in which about 100 ml. of distillate are collected in 1 hr. from 200 to 250 ml. of liquid, 2 per cent. sodium hydroxide, and 3 g. of Devarda's alloy show accurate results. With up to 50 mg. of ammonia, collection in boric acid and direct titration is satisfactory. For larger quantities, an excess of standard mineral acid, or an amount of standard acid not more than 2·5 mg.-equivalents short of the expected titre together with solid boric acid, is more reliable. L. A. D.

Factors Affecting Constancy of Analytical Weights. A. Craig (*Anal. Chem.*, 1947, 19, 72)—Rhodium and chromium make excellent coatings for one-piece brass weights, and gold and lacquer coatings are good, although more subject to wear. Any slight gain in weight, on keeping, of brass weights coated with any of these substances, or of stainless steel weights, is negligible compared with the loss due to wear when the weights are used for analysis.

Although the best manufacturers use tin or brass fillings, two-piece weights in which the weight has been adjusted by placing lead in a cavity are common. The lead oxidises rapidly and is converted into basic carbonate; this produces an increase in weight far greater than any loss due to wear, the increase being sometimes one of several milligrams. Accordingly, weights containing lead will no longer be accepted by the National Bureau of Standards for test in any of the recognised classes of standard weights. An exception is made for Class C weights in use before 1st July, 1945. B. A.



Accuracy of Colorimetric Instruments in Dye Strength Determinations. O. L. Davies, C. H. Giles, and T. Vickerstaff (*J. Soc. Dyers and Colourists*, 1947, 63, 80–83)—A statistical examination is made of the results of a series of dye strength determinations in which three colorimetric instruments were used, a Hilger "Spekker" photo-electric absorptiometer with Ilford "Spectrum" filters and a Cambridge mirror galvanometer, a Hilger-Nutting spectrophotometer, and a Duboscq-type (Ogal) colorimeter. Eight observers of various degrees of colour-matching experience were chosen and at least five of the observers were employed in the majority of the tests. Each observer operated all three of the instruments, using five acid dyes of standard commercial quality each made up in solution in four concentrations, in distilled water. The dyes chosen were the I.C.I. dyes, Lissamine Red 7BPS, Naphthalene Orange ROS, Tartrazine NS, Solway Green GS, and Solway Ultra Blue BS. Details of the experimental procedure are given. No filters were used with the colorimeter as no great improvement in accuracy was to be gained by their use

STANDARD ERRORS (PER CENT.)

	Photo-electric Absorptiometer	Spectrophotometer	Colorimeter
Lissamine Red 7BPS	0·42	1·32	1·77
Naphthalene Orange ROS ..	1·27	0·95	2·14
Tartrazine NS	0·82	1·32	5·47
Solway Green GS	1·35	2·50	2·23
Solway Ultra Blue BS	1·12	0·66	0·85
Mean	1·00	1·35	2·49

The results are summarised in the Table above, giving the standard errors of a simple test made in the normal way with the various instruments.

The Duboscq-type colorimeter gives the least, the absorptiometer the most, consistent results. The accuracy of the results given by the latter, is, unlike those of the other two instruments, independent of the colour-matching experience of the operator.

B. A.

Reviews

ORGANIC CHEMISTRY. By Professor PAUL KARRER, University of Zurich. Translated by A. J. MEE, M.A., B.Sc. Third English Edition, Revised and Enlarged. Pp. xx + 957. Published by Elsevier Co. London: Cleaver-Hume Press, Ltd. 1947. Price 38s. net.

This revised edition of Professor Karrer's well known textbook is very welcome. The author's aim is to provide a complete and interesting account of descriptive and synthetic organic chemistry, paying special attention to naturally occurring and biochemically important substances, and in these respects the book is excellent.

Since the first English edition, more than 50 pages have been added. The sections on such subjects as petroleum, theories of fermentation, proteins, polysaccharides, steroids and auxins have been amplified and revised, while new matter has been introduced dealing with pantothenic acid, penicillin, biotin (incorrectly named biotine in the text) and other recent developments. A number of new points of stereochemical interest are noted. Reference is made to DDT and to the insecticidal action of benzenehexachlorides, although no mention is made of the active γ-isomeride. A noticeable omission is any account of the recent development of new syntheses from acetylene, information about this having, perhaps, been released too late for inclusion.

Another improvement has been the introduction of sections on the electronic nature of bonds, on the mesomerism (resonance) of benzene and of triphenylmethane dyes and (in an appendix) on the electronic formulation of onium compounds. Although these additions are valuable, it is unfortunate that the application of these conceptions has not been indicated at many other points. To the modern student the electronic theory should provide interpretations of many of the familiar facts of organic chemistry. For example, it throws new light on the special features of the carboxyl group, of the CO group in esters and amides, and of the behaviour of compounds such as ethyl acetoacetate, guanidine, pyrrole and many others.

There are one or two respects in which the reader may find the symbols used ambiguous or confusing, when, for example, a co-ordinate linkage is represented in different places by the conventional arrow and as a single bond with charges, or the use on p. 64 of the same symbol for a lone pair of electrons and for a negative charge in adjacent formulae. This could be avoided by ringing the charge sign thus: ⊖

Nevertheless, this book is one of the best text-books of general descriptive organic chemistry in the English language. The production is admirable, there are remarkably few errors, and the price is actually lower than that of the pre-war first edition.

G. M. BENNETT

THE METHODS OF CELLULOSE CHEMISTRY. By CHARLES DORÉE, M.A., D.Sc., F.R.I.C. 2nd Edition. Pp. 543. London: Chapman & Hall, Ltd. 1947. Price 42s. 0d.

The first edition of this work was published in 1933, and by reason both of its subject matter and its high standard, it at once assumed the status of a standard work on this branch of cellulose chemistry. Numerous advances have, of course, taken place in the intervening years, but, as the author points out, these have been concerned with matters of detail rather than with the fundamental principles of the methods. Consequently, the general plan of the book remains unchanged, and most of the methods given previously have been retained, although they have been brought up-to-date.

On the other hand, where revision has been considered necessary, it is often on an extensive scale, and numerous additions have also been made. This applies particularly to the sections concerned with the simple and mixed esters and with the determination of molecular weights and structures. Additional chapters treat of the general properties of the esters and ethers of cellulose, and the third part of the book (dealing with the so-called "compound celluloses") has been improved by the inclusion of a fuller treatment of testing methods for wood pulp for paper making and viscose manufacture. It is desirable, however, to emphasise that the methods dealt with in this book are those of a chemical nature (including, however, viscosity measurements); evaluations of physical characteristics (such as strength and colour) of commercial pulps are not discussed. The interest in recent developments in wood lignins and plant pectins is reflected in the revised sections on these subjects; these have been largely rewritten.

If a minor point may be mentioned without conveying a disproportionate sense of criticism, it is that there is a tendency to describe several methods having the same purpose without giving any advice as to which is to be preferred and why. A particularly noticeable example of this occurs with the estimation of cellulose, where seven methods are described in detail—and the reader is left to take his choice. Similarly with lignin, for which eight methods are first given, followed by two alternative improved methods based on a preliminary hydrolysis with dilute sulphuric acid. Some critical comments from the author, with drastic pruning of methods that are open to doubt, would have saved much space and have been more helpful to the reader.

The stated object of the book is, however, to give full working details of the best chemical methods available for the isolation, experimental investigation and analysis of cellulose and its associated compounds. It undoubtedly does this,—fully, clearly, and with accuracy. It is therefore to be recommended thoroughly to all interested in this branch of work, and in particular to the analyst seeking information and working details concerning the specialised methods involved. JULIUS GRANT

INDUSTRIAL EXPERIMENTATION. By K. A. BROWNLEE, M.A. Second Edition. Pp. 121. London: H.M. Stationery Office. 1947. Price 2s. 6d.

This clear and succinct exposition of the application of statistics to industrial experiments has evidently been found to meet a real need, for the first edition (see ANALYST, 1946, **71**, 599) has been followed by a second within a year. Thirty-five pages have been added, largely as the result of rewriting the introduction and including chapters on "incomplete block" designs and on confounding. The revised version of the introduction should be even more successful in making the basic statistical principles clear to the uninitiated than was the original, but it is perhaps doubtful whether any whose knowledge of the subject is wholly derived from this book will ever dare to use the more complicated experimental designs described in the two new chapters. Chapter XII now contains a brief but very useful description of the method by which an analysis of variance can be made to yield information about the nature of the relation between two variables—a problem of frequent occurrence. A number of printers' errors, albeit minor, in the new sections appear to indicate undue haste in their preparation; this, however, may be a tribute to the speed with which the first edition has been sold. I look forward to the speedy appearance of a third edition in which these small blemishes are removed, and I reiterate the suggestion that the author should take the opportunity to include therein some discussion of the necessity for random sampling and the errors that may be introduced by lack of appreciation of the point. ERIC C. WOOD

OCTOBER, 1947 VOL. 72, No. 859

THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

An Ordinary Meeting of the Society was held on Wednesday, October 1st, 1947, at 6 p.m., in the Chemical Society's Rooms, Burlington House, W.1. The President, Mr Lewis Eynon, was in the Chair. The following papers were presented and discussed—"The Determination of Small Amounts of Hexachlorocyclohexane (Benzene Hexachloride)," by Bernard H. Howard; "Iodimetric Methods of Estimating Peroxide Oxygen," by J. H. Skellon and E. D. Wills; "A Study of the Cobalt-Ferricyanide Reaction with relation to the Determination of Cobalt in Steel," by B. Bagshaw and J. D. Hobson.

NEW MEMBERS

Allan Adair, B.A., B.Sc., D.Phil. (Oxon.); Thomas Ralph Andrew, B.Sc., A.R.I.C., Kenneth Vincent Bloomfield, B.Sc. (Lond.), F.R.I.C.; Frederick Percy Handisyde; Rupert Eardley Robinson, F.R.I.C., A.M.C.T.; Percy Smith, A.R.T.C.

DEATHS

We regret to have to record the deaths of

Frederick Alfred Mason.
Alfred Sherlock.
Herbert Procter Smith.

Polarographic Determination of Lead in Foodstuffs

By F. R. JONES and (MISS) D. M. BRASHER

A polarographic method has been developed for the determination of lead in foodstuffs. It is simple and convenient and does not require preliminary separation of the lead; the conditions of electrolysis are made such that trace metals (Fe, Sn, Zn, As, Cu) do not interfere with the lead wave. The method could be used as an alternative to chemical analysis in routine work.

Few references in the literature relate to the polarographic estimation of lead in organic materials. Yosida[1] devised a method based on the preliminary separation of lead electrolytically as PbO_2; Teisinger[2] estimated lead in blood by adding hydrochloric acid and recording the current - voltage curve directly; Forche[3] improved the accuracy of this method by first decomposing the organic matter with sulphuric, nitric and perchloric acids, evaporating the resulting solution to dryness and submitting a solution of the residue in alkaline tartrate to polarographic analysis.

These methods suffer from disadvantages, the first because of the necessity for preliminary separation of lead, the second through interference of tin, and the third through interference of both tin and iron. A further difficulty is that a satisfactory lead wave is not normally obtained from the electrolysis, in the polarographic cell, of the sulphuric acid solution resulting from the wet destruction of foodstuff; this is because of interference by residual organic matter.

As an outcome of the work herein reported, the difficulties can be overcome as follows:—

(*a*) *Interference of residual organic matter*—Complete destruction of organic matter has been found essential to the production of a horizontal current - voltage curve in the absence of trace metals. This is accomplished by the use of perchloric acid.

(*b*) *Interference of ferric iron*—This produces a wave at a potential more positive than that of lead, and in samples containing a relatively large quantity of iron the pronounced oscillations that follow the iron wave, and the accompanying maxima, make it difficult or impossible to measure the lead wave. Iron, therefore, is first reduced with hydroxylamine hydrochloride.[4] Investigation has shown that reduction is incomplete in solutions of acid normality greater than 2·5, but is effective, up to 2000 p.p.m. of iron in the sample taken, if the normality is suitably adjusted. The resulting ferrous ion is reduced at the dropping mercury electrode at about −1·2 volt, and therefore does not interfere with the lead wave, which occurs at −0·5 volt.

(*c*) *Interference of tin*—In the stannous state (after treatment with hydroxylamine hydrochloride) in sulphuric acid solution of concentration approaching 2·5 N, tin produces a wave at − 0·45 volt, that is, at a potential almost identical with that of lead. Investigation has shown, however, that, even with a concentration of 300 p.p.m. of tin in the sample taken, the tin wave can be eliminated without effect on the lead wave, by adjustment of the *p*H to a value between 2·5 and 3·0 by addition of sodium hydroxide. At values much above 3·0, for example at *p*H 5·5, the height of the lead wave is considerably reduced. The control of *p*H is very simply effected with the aid of two indicators which also act as maximum suppressors.

Other trace elements likely to be present in foodstuffs do not interfere with the lead wave. Zinc and arsenic are reduced at −0·7 and −1·1 volt respectively, and copper is reduced at zero potential in presence of hydroxylamine hydrochloride, which provides sufficient chloride ions to stabilise the potential of the anode (a pool of mercury in the reaction cell) at that of a calomel half-cell.

With these precautions, a satisfactory lead wave can be obtained from the sulphuric acid solution resulting from the wet destruction of the material, but the value of the diffusion current is affected by the viscosity of the electrolyte. It has been shown[5] that, in solutions of sulphuric acid and of sodium sulphate, the diffusion current of lead, and of other ions, is inversely proportional to the square root of the viscosity of the electrolyte. The electrolyte, prepared as indicated above, consists mainly of a solution of sodium sulphate, of which the concentration—and hence also the viscosity—may vary largely depending upon the quantity of sulphuric acid remaining after destruction of the sample and upon its subsequent dilution.

The diffusion current is very simply corrected for viscosity with the aid of the curve given in Fig. 2, and the corrected value is referred to a calibration curve, Fig. 3, to obtain the concentration of lead in the solution electrolysed.

The amount of lead that can be determined by this procedure is limited by its solubility in sulphuric acid, but with a 5-g. sample containing up to 40 p.p.m. no loss of lead has been experienced.

METHOD

APPARATUS—

The Heyrovský cell used for electrolysis is shown, in position under the dropping mercury electrode, in Fig. 1. The water-bath in which it stands is capable of being lowered from this position and the bung carrying the electrode is fitted with a short glass tube which, by dipping into the water when the bath is raised, isolates the contents of the cell from the atmosphere. A second glass inlet (2) is fitted through the bung.

In the analyses reported below, hydrogen, used for removing air from the cell and its contents, was generated electrolytically. Polarograms were recorded with a Heyrovský Micro-Polarograph (Nejedly, Prague) modified by incorporation of a circuit for compensation of residual current. The photographic paper used in the recording drum had a grid printed photographically upon it so that the current - voltage curve and the grid both appeared on development. Readings of current and potential were made directly.

PROCEDURE—

(1) *Preparation of the solution for electrolysis*—Destroy 5 g. of the sample in a Kjeldahl flask in the usual way with sulphuric and nitric acids. Note the volume of sulphuric acid used; it should not exceed 2 ml.* When destruction is apparently complete, add about 50 ml. of water and boil until fumes are evolved. Add 3 drops of perchloric acid, heat and add more perchloric acid if a bright yellow colour is not developed in about 1 minute; add

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* The concentration of lead in the solution electrolysed may be too small for the accurate measurement of the lead wave if more than 2 ml. of sulphuric acid are used with a sample of low lead content. Destruction of some foods can be accomplished with less than this quantity of acid if the sample is first allowed to stand overnight in contact with nitric acid.

about 50 ml. of water and boil until fumes are evolved. The residual acid should now be clear and colourless. Transfer to a measuring cylinder with the aid of two or three small quantities of water, each boiled in the flask, and bring the diluted acid to a volume, V ml., which must be near to, but not greater than, 15 times the volume of sulphuric acid used for the destruction of the sample. Determine its normality, S, by titration of a small portion with 0·5 *N* sodium hydroxide; it should be less than 2·5 (≡ 1·25 *M*).

Transfer 10 ml. of the acid solution to the polarographic cell, add about 0·1 g. of hydroxylamine hydrochloride, connect the side-tube (Fig. 1 (1)) to the hydrogen supply, place the cell over a bunsen and boil the solution, very gently to minimise loss by evaporation. After 5 minutes, bubble hydrogen through the solution and place the cell in the water bath in the lowered position under the dropping mercury electrode. Add 3 drops each of bromophenol blue and thymol blue indicators, B.D.H., followed by 5 *N* sodium hydroxide from a 10-ml. graduated pipette until the colour of the solution is just violet; add 2·5 *N* sulphuric acid from a 1-ml. graduated pipette until the colour is just yellow. Note the combined volume of alkali and acid added (v ml.). The volume of indicators added can be neglected as it approximates to that of the water lost by evaporation. The volume of the solution electrolysed is thus (10 + v) ml.

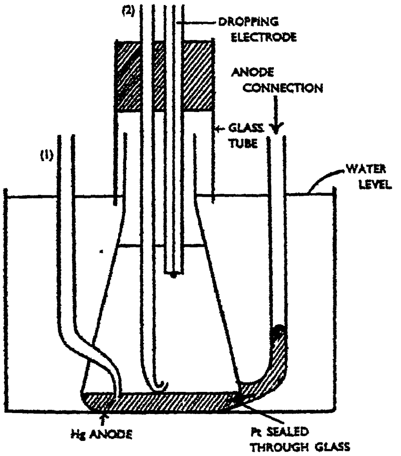

Fig. 1. Electrolytic Cell for Determination of Lead

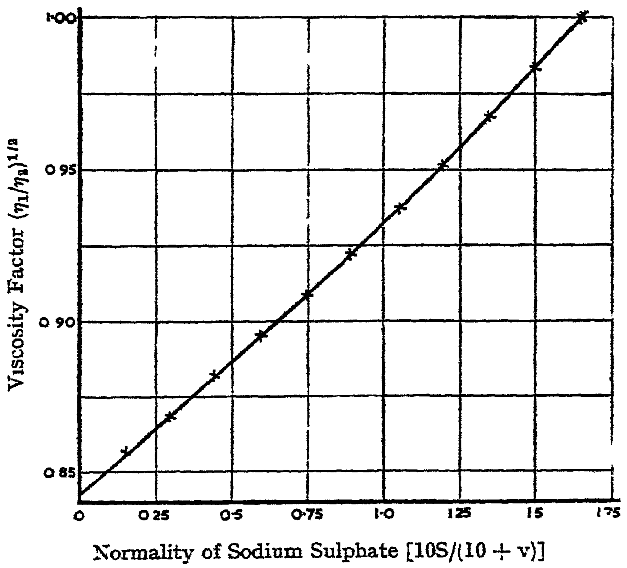

Fig. 2. Viscosity Factor.

Raise the water-bath and cell to the position shown in Fig. 1, transfer the hydrogen supply to tube (2) and introduce sufficient mercury through tube (1) to cover the tip of this tube and the platinum anode. Adjust the level of water in the bath so that the end of the glass tube is immersed. Continue the passage of hydrogen for a further 10 minutes, and meanwhile adjust the temperature of the bath. Record the polarogram under standardised conditions of rate of flow of mercury and rate of change of applied potential. It is only necessary to record between —0·2 and —0·7 volt.

(2) *Correction for viscosity*—The graph of the viscosity factor, Fig. 2, was constructed from experimentally determined viscosities (relative to water) of solutions of sodium sulphate; η_2 is the viscosity of the 1·67 *N* solution which results from the neutralisation to *p*H 3·0 of 2·5 *N* sulphuric acid with 5 *N* sodium hydroxide and which was used for the construction of the calibration curve, Fig. 3; η_1 is the viscosity of a solution of sodium sulphate of given normality. The values of $(\eta_1/\eta_2)^{\frac{1}{2}}$ lie on a smooth curve which is not quite linear.

To correct the diffusion current as obtained from the polarogram for the variation of the viscosity of the electrolyte from the standard value, η_2, calculate the normality of sodium sulphate in the solution electrolysed:

$$\text{Normality} = 10\,\text{S}/(10 + \text{v})$$

where S = normality of the dilute acid solution as found by titration, and v = volume of reagents added to 10 ml. of this acid solution, and refer the value found to Fig. 2. Multiply the diffusion current measured at the half-wave potential of lead, *viz.*, — 0·5 volt, by the value of $(\eta_1/\eta_2)^{\frac{1}{2}}$ corresponding to the normality of the sodium sulphate. In determining the diffusion current from the polarogram no correction for variation of drop time is necessary; the drop time is sufficiently constant under standardised conditions.

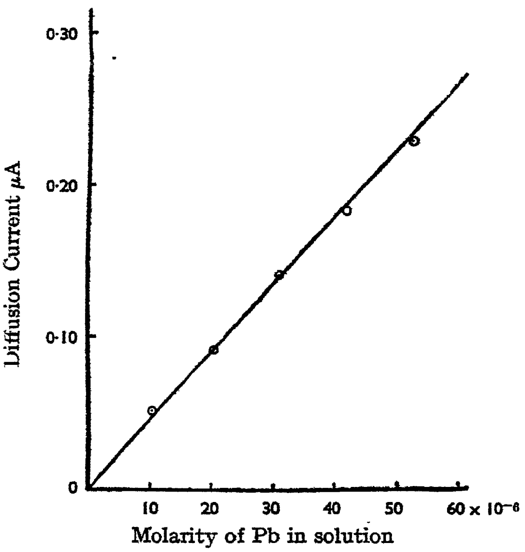

Fig. 3. Calibration Curve of Pb in Na_2SO_4 solution (16·7 *N*) at *p*H 3.

(3) *Calculation of lead content*—The calibration curve, Fig. 3, was constructed from polarograms of solutions of known lead content; for each determination 10 ml. of a solution of lead in 2·5 *N* sulphuric acid was treated with hydroxylamine hydrochloride and neutralised exactly as described above. The normality of the solution of sodium sulphate electrolysed was 1·67 (≡ molarity 0·835); its molar concentration of lead was calculated from the known amount of lead in the acid solution. The points on the curve in Fig. 3 should lie on a straight line passing through the origin.

To calculate the lead content of the sample analysed, refer the diffusion current, corrected for viscosity, to the calibration curve to obtain the molarity of lead in the solution electrolysed. If M be this molarity, the weight of lead in grams is:

0·207 M (10 + v) in the solution electrolysed,

and 0·0207 MV (10 + v) in the sample,

where V = the volume in ml. to which the sulphuric acid resulting from the destruction of the sample was diluted.

If 5 g. of material was destroyed the lead content is:

4140 MV (10 + v) p.p.m.

RESULTS

The accompanying table shows results obtained by this method on samples of canned meat and fish, the lead contents of which were first determined by chemical analysis with an accuracy of ±0·5 p.p.m. Known quantities of tin and lead were added and the table shows the quantity of tin added to, and the total lead present in the sample taken for analysis. The last column shows the molarity of lead in the solution electrolysed.

The standardised conditions of electrolysis were:

Temperature of water in bath	25° C.
Height of mercury column above tip of capillary ..	60·0 cm.
Rate of flow of mercury	1·56 mg. sec.$^{-1}$
Sensitivity	1/1
Potential applied to drum	2·0 volt
Rate of change of applied potential	0·2 volt min.$^{-1}$
Counter residual current	set as required

The results, which show that the method is not affected by the presence of tin up to 115 p.p.m., are considered to be sufficiently accurate for all normal requirements. Kolthoff and Lingane[6] state that the accuracy of polarographic analysis under ordinary conditions is of the order of ±2 per cent. in the concentration range 10^{-2} to 10^{-4} molar (16,000 to 160 p.p.m. lead) and ±5 per cent. in the range 10^{-4} to 10^{-5} molar (160 to 16 p.p.m. lead).

The table shows that the molarities are in the range 10^{-5} to 10^{-6}, these low values being due to the high atomic weight of lead; larger relative errors are to be expected with molarities of the order of 10^{-6}, for the wave heights are very small, *e.g.*, about 3 mm. in the first result quoted.

TABLE

Tin added p.p.m.	Lead content (total) p.p.m.	Lead determined by polarograph p.p.m.	Error p.p.m.	Error per cent.	Molarity of lead in solution electrolysed
0	5	5	0	0	$4\cdot0 \times 10^{-6}$
35	5	7	+2·0	+40·0	$4\cdot7 \times 10^{-6}$
35	8	10	+2·0	+25·0	$6\cdot2 \times 10^{-6}$
80	12	13	+1·0	+8·3	$9\cdot5 \times 10^{-6}$
35	18	18·5	+0·5	+2·8	$1\cdot1 \times 10^{-5}$
35	20	21·5	+1·5	+7·5	$1\cdot1 \times 10^{-5}$
0	23	23	0	0	$1\cdot5 \times 10^{-5}$
35	29	26·5	−2·5	−8·6	$1\cdot7 \times 10^{-5}$
35	32	32	0	0	$1\cdot8 \times 10^{-5}$
35	32	35	+3·0	+9·4	$1\cdot9 \times 10^{-5}$
80	32	27·5	−4·5	−14·1	$1\cdot7 \times 10^{-5}$
115	41	41·5	−0·5	+1·2	$2\cdot9 \times 10^{-5}$

The authors wish to thank Messrs. J. Lyons & Co., Ltd., in whose laboratories the work was conducted, for permission to publish this paper.

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June, 1947

The Determination of Small Amounts of Hexachlorocyclohexane (Benzene Hexachloride)

BY BERNARD H. HOWARD

(*Read at the Meeting of the Society on Wednesday, October 1st*, 1947)

INTRODUCTION

HEXACHLOROCYCLOHEXANE, benzene hexachloride, is a mixture of isomers of which the γ-isomer ("Gammexane") is strongly insecticidal. The normal commercial product contains about 13 per cent. of this isomer, to which almost the whole of the insecticidal activity is due. The toxicity towards mammals is also mainly associated with the gamma isomer.[1] Although biological, *i.e.*, insecticidal, methods of assay are known,[2] chemical methods were required. Such a method should, as far as possible,

(1) be readily applicable to grain and other foodstuffs and to dust and spray deposits on surfaces or fabrics,
(2) be applicable to the routine testing of numerous samples in a chemical laboratory of modest equipment,
(3) be sensitive to a few micrograms of benzene hexachloride,
(4) be specific for benzene hexachloride, especially to distinguish it from other insecticides such as DDT, and
(5) distinguish between the gamma and other isomers present.

The method here described complies with conditions (1), (2) and (3). Point (4), which requires a closer examination of the properties of the substances concerned, has not been fully covered at this stage and is undergoing further investigation. With regard to (5), there appears to be no chemical method available for distinguishing the isomers.

A knowledge of the proportion of gamma isomer in the original preparation is necessary to estimate the insecticidal activity of the amount of benzene hexachloride revealed by chemical methods.

PRELIMINARY EXPERIMENTS

A. A method involving the separation of trichlorobenzenes with alkali, followed by their intensive nitration, has been described.[2] The nitro-chloro compound decomposes, when boiled with alkali, to give a brightly yellow-coloured nitro-phenol. In the absence of a photo-electric absorptiometer, which was employed by the authors of the above-mentioned method, the possibility of using simple comparisons with Nessler glasses was tested. However, the colour did not appear strong enough to show less than about 0·1 mg. The method was not proceeded with as it was not considered to satisfy the above general conditions (2) and (3).

B. Although a method of determining total chlorine, such as that of Carius, might seem potentially more sensitive than the method removing only half the total chlorine; later to be described, this advantage was considered to be outweighed by the difficulties and hazards of the Carius technique. Furthermore, in presence of much foreign matter a large amount of nitric acid would be required: this is to be avoided because A.R. nitric acid contains up to 1 mg. of chloride per litre.

C. Rough experiments suggested that benzene hexachloride is inert to ammonia, silver nitrite and potassium cyanide, the chlorine atoms appearing not to undergo readily any replacement reactions.

D. Previous reports that benzene hexachloride reacts readily with alkalis to yield trichlorobenzenes and alkali chlorides were confirmed and, since this reaction readily lends itself to compliance with requirements (1) and (2) above, it was examined further.

Van den Linden[3] reported that with alcoholic potash the reaction

$$C_6H_6Cl_6 + 3OH' = C_6H_3Cl_3 + 3Cl' + 3H_2O$$

is quantitative. This was confirmed, but in practice alcoholic alkali was found to have two serious disadvantages—(*a*) Caustic potash, even of A.R. quality, contains up to 0·01 per cent. of chloride. Two ml. of 10 per cent. solution, the amount of alkali used in the preliminary experiments, would therefore contain chloride equivalent to approximately 50 μg. of benzene hexachloride. Even this high blank figure tended to increase when the solution was stored in the laboratory atmosphere. (*b*) In the determination of benzene hexachloride in foods, the final extract for reacting with alkali consisted of a solution of the insecticide in vegetable fat. Enough alkali had to be added to decompose all this fat: sometimes 5 ml. or even more of the 10 per cent. solution. In some instances the chloride in the reagents was much greater than that derived from the benzene hexachloride. The use of alkali was therefore abandoned.

E. Organic bases, that might be freed from chloride by distillation, were next tried as dehydrochlorinating agents. Pyridine appeared to be without effect, but monoethanolamine was satisfactory. After distillation from alkali, the chloride content of this reagent was less than 5 μg. per ml., corresponding to about 15 μg. of benzene hexachloride per ml. It was also found that, under the conditions used, this reagent is without effect on fats. The presence of fats, therefore, does not necessitate the use of a large amount of reagent. Enough must be added, of course, to neautralise any free acid in the fat. To test the reaction, various amounts of benzene hexachloride were decomposed by various amounts of monoethanolamine in stoppered tubes immersed in a boiling water bath and the liberated chloride was titrated by Volhard's method.

To investigate the applicability of the method to all forms in which benzene hexachloride might be met in practice, decomposition tests were performed on (*a*) the substance alone, as would be found in dusts or smoke deposits, (*b*) solutions in mineral oils, as would be found in spray deposits, (*c*) solutions in vegetable oils, as would be obtained when foodstuffs are extracted to isolate the benzene hexachloride. Shell oil P.31, a non-volatile paraffin oil, was the mineral oil used. Three samples of arachis oil were available and it was assumed that the reaction in other vegetable oils would be similar. The benzene hexachloride used was recrytstallised material containing 95 per cent. of the gamma isomer. The mixture of isomers met in practice behaves very similarly with dehydrochlorinating reagents. The larger quantities were weighed direct into the reaction tubes; the smaller quantities were obtained by evaporating the appropriate volumes of a 0·100 per cent. solution of the substance in ether.

Each result shown in the table is the mean of at least three tests.

DECOMPOSITION OF BENZENE HEXACHLORIDE BY MONOETHANOLAMINE

In tests 7 and 8 the monoethanolamine was added as a 1 per cent. aqueous solution; in tests 10, 11, 12, 20, 27 and 28 as a 10 per cent. solution in chloride-free glycerol. The glycerol was used merely as a solvent, as experiments suggested that when several milligrams of solid benzene hexachloride were used the reaction with the small volume of monoethanolamine did not always proceed to completion, because of insufficient mixing. Of the vegetable oils, (1) and (3) had low acid values, about 0·3, whilst (2) had a very high acid value, about 40.

Test No.	Benzene hexachloride taken mg.	Monoethanolamine used ml.	Added matter	Time in water bath min.	Decomposition per cent.
1	100	2	—	15	98
2	100	2	—	30	98
3	100	2	—	60	102
4	100	2	—	180	103
5	100	0·1	—	60	80
6	1·0	0·1	—	60	99
7	5·0	0·1 in 10 ml. water	—	60	74
8	5·0	0·05 in 5 ml. water + 3 ml. alcohol	—	60	62
9	100	0·1 in 2 ml. alcohol		30	87
10	100	0·1 in 1 ml. glycerol + 2 ml. alcohol		60	90
11	5·0	0·1 in 1 ml. glycerol + 2 ml. alcohol		60	100
12	1·0	0·1 in 1 ml. glycerol		60	100
13	100	1	P.31 oil 5 ml.	30	87
14	100	1	” 5 ”	60	101
15	100	0·5	” 5 ”	60	92
16	100	0·1	” 5 ”	60	43
17	5·0	0·1	” 5 ”	30	74
18	5·0	0·1	” 5 ”	60	94
19	5·0	0·1	” 0·5 ”	60	104
20	1·0	0·1 in 1 ml. glycerol	” 0·5 ”	60	102
21	100	0·5	Arachis oil (1) 5 ml.	30	100
22	100	0·1	” (2) 5 ”	60	20
23	5·0	0·1	” (3) 2 ”	60	76
24	5·0	0·2	” (3) 2 ”	60	104
25	1·0	0·1	” (3) 0·5 ”	60	102
26	1·0	0·2	” (3) 2 ”	60	101
27	1·0	0·1 in 1 ml. glycerol	” (3) 0·5 ”	60	41
28	1·0	0·2 in 2 ml. glycerol	” (3) 2 ”	30	17
29	300 mg. DDT.	2	—	30	97

These results show—(i) That amounts of benzene hexachloride from 1 to 100 mg. can be determined accurately either alone or dissolved in mineral or vegetable oils, by decomposing with small amounts of monoethanolamine. In tests 5, 9 and 10, 80 to 90 per cent. decomposition was produced by 1·6 times the amounts of monoethanolamine required by calculation. Where small quantities are being determined, it is obviously advantageous to use very small amounts of the reagents. (ii) The presence of oils necessitates the use of more reagent (*cf.* tests 5, 16; 18, 23). (iii) The aqueous solution of monoethanolamine gives low results, and the glycerol solution does not seem to be necessary for complete reaction of the smaller amounts of benzene hexachloride (test 6). (iv) The low results obtained from arachis oil solutions with the glycerol solution of the reagent are surprising (tests 27, 28). The addition of glycerol renders the mixture of oil and monoethanolamine homogeneous, and a quicker reaction was expected on this account. (v) Under similar conditions the benzene hexachloride is more rapidly decomposed when dissolved in arachis oil than in mineral oil. (vi) The highly acid arachis oil No. 2 prevented the decomposition of the benzene hexachloride dissolved in it (test 22). That decomposition occurred at all is remarkable, as enough acid was present in the oil to neutralise all the monoethanolamine added. (vii) It is known that DDT is dehydrochlorinated by means of alkali under the same conditions as is benzene hexachloride. The test with DDT, No. 29, indicates that its behaviour with monoethanolamine also is similar to that of benzene hexachloride. The decomposition of DDT by organic bases has already been noted.[7] ✓

For smaller amounts of benzene hexachloride, 20 to 200 μg., both alone and dissolved in the oils (except the very acid arachis oil), and with quantities of monoethanolamine similar to those shown in the above table, the decomposition was still sufficiently close to the theoretical for practical use. The chloride was determined turbidimetrically in these experiments.

F. In practice it is essential to be able to determine benzene hexachloride when inorganic chlorides may be present, *e.g.*, in foodstuffs. Extraction with ether gives a material free from inorganic chloride, as the following tests prove. (i) Two g. of sodium chloride and ammonium chloride were each shaken with 50 ml. of ether. After filtering and evaporating, no turbidity was detectable on addition of silver nitrate. (ii) 100-g. samples of several foodstuffs not contaminated with benzene hexachloride were treated with ether, and the extracts tested in the manner described below. In no case did the final solution show an appreciable turbidity with silver nitrate solution. The materials thus shown to give negligible blanks were maize, barley, wheat, ground nuts, flour, dried yeast, fish meals, meat and bone meal, bitter almonds, dried apricots and milk.

The method finally adopted is as follows.

METHOD

REAGENTS—

(1) *Distilled water:* chloride-free. (2) *Ether.* (3) *Monoethanolamine:* re-distilled from caustic soda. (4) *Nitric acid:* 10 per cent. of A.R. concentrated acid in water. (5) *Sodium chloride solutions:* a 0·06 per cent. solution is diluted tenfold before use, to give a standard solution for turbidimetric determination of chloride; 1 ml. of the diluted solution contains as much chloride as is liberated from 0·1 mg. of benzene hexachloride by dehydrochlorination. (6) *Silver nitrate solution:* about 0·1 *N*, in a dropping bottle. (7) *Silver nitrate solution, standard:* 0·01 *N*. (8) *Ammonium thiocyanate,* and *ferric alum solutions,* for determination of chloride by Volhard's method; or solution of *dichlorofluorescein* for determination of chloride by Fajans' method.

PROCEDURE—

(i) For foodstuffs. Extract 50 to 100 g. of the sample with ether. The tenacity with which the benzene hexachloride is held varies with the conditions of application. From grain, for example, benzene hexachloride added as a dust may be removed almost completely by two simple washes with ether, but when it has been added as an oil spray more thorough extraction is necessary. For all foodstuffs, therefore, Soxhlet extraction, until all fat is removed, is recommended.

(ii) For deposits. Wash the object well with a jet of ether. If there is any doubt as to absorption of benzene hexachloride by the object, the Soxhlet method should be adopted if possible.

Filter the ethereal solution resulting from these procedures into a boiling tube fitted with a ground-glass stopper. Evaporate the ether in a stream of air while the tube is gently warmed. Add 0·1 ml. of monoethanolamine, or, if oil is present, 0·1 ml. for every ml. of oil. (This is because the figures given in the table demonstrate that about one-tenth as much reagent as oil will react satisfactorily.)

Stopper the tube and immerse in boiling water to a depth of about 2 in. for 1 hour, with frequent shaking. A wire basket to hold about ten tubes was found convenient for holding the tubes in a can of boiling water.

Remove from the water bath after an hour and add 5 ml. of the 10 per cent. nitric acid, or, if more than 0·5 ml. of monoethanolamine has been used, add 10 ml. of acid for every 1 ml. of reagent. Add next from 15 to 20 ml. of water and, when cool, 20 ml. of ether, and shake. After settling, blow off the ethereal layer through a Werner - Schmid tube. If the aqueous layer is still cloudy repeat the ether extraction.

The oils, trichlorobenzenes, etc. having been removed by this ether extraction, the aqueous solution is in a suitable state for estimation of chloride. With 0·01 *N* reagents it was found possible to determine down to 0·2 mg. of benzene hexachloride by Volhard's method. For smaller quantities, down to 20 μg., the chloride can be determined turbidimetrically. Any faint cloudiness in the solution can be removed by filtration. Bring the volume up to the 50-ml. mark of the Nessler glass. In other glasses prepare standards containing different amounts of the diluted salt solution, and the same volumes of monoethanolamine and nitric acid as were used on the sample under test, in a volume of 50 ml. Add three drops of the silver nitrate solution to the sample and standards, stir, and compare against a dark ground after about 2 minutes.

Sensitivity—In 50 ml. of solution in a Nessler glass the eye can detect a turbidity due to 5 μg. of chloride ion, equivalent to 14 μg. of benzene hexachloride. In field experiments involving dust and spray deposits the density of the deposit has been found by collecting samples on 3 in. × 1 in. microscope slides, having an area of 19·4 sq. cm. By this method the minimum surface density detectable is 0·7 μg. per sq. cm. In tests on foodstuffs, where a sample of 100 g. might be used, this is equivalent to a concentration of 0·14 p.p.m. The method is sufficiently sensitive to measure very slight contamination of foodstuffs.

Selectivity—Many organic chlorine compounds lose chlorine in treatment with alkali, but none of these occurs naturally. Production of a positive result in the test described above, when applied to foodstuffs, is therefore evidence of contamination by organic chlorine compounds. The most likely such substance with which benzene hexachloride might be confused is DDT, and a test to distinguish the two is necessary where doubt is possible. Of the numerous colour reactions of DDT the pyridine - xanthydrol reaction[4] is simple. It is very sensitive to DDT and its analogues but gives no colour with benzene hexachloride. This could be used on a small portion of a sample to distinguish the two insecticides.

Test 29 suggests that the treatment with monoethanolamine as described in this paper would be applicable to the determination of DDT, but this has not been investigated in further detail. However, a much smaller amount of chloride is removed from DDT than from benzene hexachloride, 1 g. of the former giving 100 mg. of chloride ion and 1 g. of the latter 366 mg. The method would therefore be only about one-third as sensitive if it were applied to DDT.

SOME APPLICATIONS OF THE METHOD

(1) To grain—Wheat, contained in a beaker, had been exposed to a smoke of benzene hexachloride produced from a Gammexane Smoke Generator. It remained toxic to adult *Calandra granaria*, even after two sievings. The extract from 100 g. of the wheat was found to contain 0·5 mg. of benzene hexachloride, corresponding to 5 p.p.m. in the wheat.

(2) To dust deposits—(*a*) In an experimental chamber of 260 cu. ft. capacity 3 in. × 1 in. microscope slides were placed on the floor and on shelves, and other slides were fixed to the walls by pellets of modelling clay. A 0·5-oz. portion of a Gammexane Smoke Generator was ignited, and after 3 hours the slides were picked up by forceps and placed in the stoppered boiling tubes for removal to the laboratory. The densities of the deposit on the floor and shelves at different heights were very similar, average 20 μg. per sq. cm. The deposit on walls or ceiling was less, about 4 μg. per sq. cm.

(*b*) A ship's hold was treated with smoke generators, and samples were collected on microscope slides. The deposit on horizontal surfaces was heavy, from 20 to 220 μg. of benzene hexachloride per sq. cm.

(3) Recent investigations into the behaviour of these insecticides in the animal body, *e.g.*, the determination of DDT in the milk of cows whose diet contains the substance,[5,6] suggest further applications of the method here described, which is likely to obviate certain difficulties, due to the presence of milk fat, previously encountered in this field. The method might also be adapted to the determination of the insecticides in the blood.

SUMMARY

A method for the analysis of insecticidal deposits, or of residues in foods, without the use of elaborate equipment was required. After examination of alternatives, a method depending upon extraction with ether followed by dehydrochlorination with monoethanolamine, and subsequent determination of the separated chloride, was developed. The scope and limitations of the method are discussed.

The author wishes to thank Imperial Chemical Industries for the gift of the "Gammexane" used in these experiments, Dr. E. E. Turtle for help in preparing this paper and W. McAuley Gracie, Esq., Director of Infestation Control, for permission to publish.

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Discussion

The President said the author had remarked that commercial Gammexane usually contained about 13 per cent. of the gamma isomer. Was this proportion sufficiently constant to enable a reasonably reliable estimate of insecticidal potency to be based on determinations of total benzene hexachlorides?

Mr. J. H. High asked if it was possible to take advantage of physical properties of the isomers to effect, not necessarily a complete, but at least a partial, separation, and to estimate the relative amounts of the isomers from differences in the physical properties before and after the separation. He also asked if the reproducibility of the results obtained with alkali was good; if it was, the presence of a constant proportion of chloride might not be a fatal disadvantage.

Mr. F. C. Hymas asked if any control experiments had been made to determine "blanks" on prepared foods. It was to be expected that blank determinations on grains and on inorganic chlorides would yield negative results, but there was some reason to think that blanks on prepared foods containing added halides might be appreciable.

Mr. Howard, in reply to the President, said that although the proportion of active isomers in commercial benzene hexachloride varied with the conditions of manufacture, it was likely to be fairly constant under the same conditions. But he understood that the material was now being marketed with higher proportions of the active constituents. In reply to Mr. High, he said that the different isomers did not differ much in physical properties. He had heard that work had been done in America on discrimination between the isomers by means of chromatography and infra-red spectrography. The blank figure resulting from the use of alkali had been found to be somewhat variable, and this had led to uncertainty in the true figure. The author said that he had not made determinations of the kind mentioned by Mr. Hymas, on prepared foods.

The Determination of Nitromethane in Air

By H. N. WILSON and W. HUTCHINSON

In the manufacture of nitromethane it is necessary to ensure that the atmosphere contains only minute quantities of the nitro-body, which has toxic properties. A method was therefore required to determine nitromethane concentrations of the order of 1 or 2 mg. per litre in the atmosphere where work on nitroparaffins was in progress.

Preliminary work showed that nitromethane could be determined in 0·2 N sulphuric acid by the polarograph, giving a well defined step with a half-wave potential of —0·7 to —0·8 volt against the saturated calomel electrode. Suitable solutions were made up and polarographed with a Cambridge instrument at a sensitivity of 1/700. Fig. 1 shows the step height against concentration at this sensitivity. It was found that one division of step height ≡ 16·8 mg. of nitromethane per litre of 0·2 N sulphuric acid.

Recovery of nitromethane in air by passage through gas absorption bottles containing 0·2 N sulphuric acid was unsatisfactory and was abandoned in favour of a technique using an evacuated winchester. This employs a winchester fitted with a two-holed rubber bung carrying a 50-ml. tap funnel and a right-angled glass tube with tap for connection to a good water pump. To test this method, known amounts of nitromethane contained in sealed glass bulbs were introduced into the winchester, the volume of which was known. The air was removed by the water pump, the pressure being measured by a mercury manometer. The glass tap to the pump was then closed, the pump disconnected and the bulb inside the winchester broken. The winchester was allowed to stand under reduced pressure for 24 hours to ensure complete vaporisation of the nitromethane. Air was then admitted until atmospheric pressure was reached, the tap closed and the winchester allowed to stand a further

Experiment	Nitromethane added mg.	Concentration in atmosphere at 20° C. and 760 mm.		Nitromethane found mg.	Percentage recovery at 20° C.
		mg./litre	% by volume		
1	36·2	13·7	0·475	34·4	95·0
2	24·8	9·4	0·320	23·6	95·2
3	18·5	7·0	0·238	17·6	95·1
4	11·0	4·2	0·143	10·5	95·5
5	6·20	2·3	0·079	5·9	95·2
6	4·762	1·8	0·061	4·514	94·8
7	3·824	1·45	0·050	3·606	94·3
8	3·018	1·13	0·038	2·852	94·5
9	1·268	0·478	0·016	1·192	94·0
10	0·621	0·224	0·008	0·578	93·0

2 hours to ensure thorough mixing of the air with the nitromethane. Fifty ml. of 0·2 *N* sulphuric acid were introduced by cooling the winchester slightly and allowing the acid to be drawn in *via* the tap funnel. The winchester was well shaken and allowed to stand 20 minutes and the 0·2 *N* sulphuric acid containing the nitromethane polarographed without de-oxygenating.

The data on p. 432 indicate the recovery of nitromethane from air, in concentrations varying from about 0·5 to 0·01 per cent. by volume.

It thus appears that under these conditions the dilute sulphuric acid in equilibrium with the atmosphere at 1 atmosphere pressure absorbs 95 per cent. of the nitromethane present and this figure must be taken into account in the analysis of samples of air.

PROCEDURE—Evacuate a winchester of known volume to about 30 mm. of mercury pressure, measuring the pressure with a manometer. Then open it in the atmosphere to be tested, taking the temperature at the same time. Introduce 50 ml. of 0·2 *N* sulphuric acid by cooling the winchester slightly and allowing the acid to be drawn in. Shake well and allow to stand for 30 minutes. Polarograph the sulphuric acid solution, without de-oxygenating, at a suitable sensitivity, *e.g.*, 1/700 or 1/70, over the voltage range —0·3 to —1·3 volt against the saturated calomel electrode.

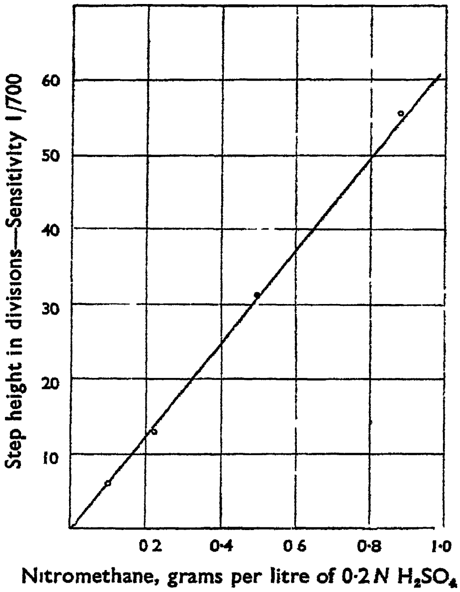

Fig. 1

Prepare a graph similar to Fig. 1 by dissolving known weights of pure nitromethane in a known volume of 0·2 *N* sulphuric acid and polarographing the solutions under the same conditions. Plot grams or milligrams of nitromethane per litre of 0·2 *N* sulphuric acid against the step height. From the graph, the volume of 0·2 *N* H_2SO_4 used and the volume of the air sample taken, calculate the proportion of nitromethane in the atmosphere, allowing also for the 95 per cent. recovery.

Note—Other nitroparaffins present will react similarly to nitromethane. If some other nitro-body is expected to predominate, a new graph must be prepared.

SUMMARY

A method for the determination of small quantities of nitromethane in air has been described. The nitromethane is absorbed in 0·2 *N* sulphuric acid and the concentration in this determined with a polarograph.

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IMPERIAL CHEMICAL INDUSTRIES LTD.
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The Estimation of the Volatile Matter Content of Propellant Explosives

Part 3.* The Estimation of Acetone

By T. G. BONNER

ACETONE is widely employed in the manufacture of various types of cordite (*i.e.*, propellants, containing both nitrocellulose and nitroglycerine). The acetone is used in optimum admixture with water to facilitate the formation of a homogeneous solution of the constituents of the cordite, which is necessary to ensure uniformity of product. The presence of the solvent in the final product is, of course, undesirable, and the last stage of manufacture is designed to remove as much of it as possible by "stoving" at an elevated temperature. This removal is never complete and in order to assess the effect of the residual acetone on ballistic properties, particularly in relation to variations in the acetone content with changing temperature and humidity, an accurate method of estimation is of paramount importance.

Various workers[1,2,3] have reported attempts to determine the residual acetone content, but no evidence is advanced that the methods give absolute figures and in general the results are quite arbitrary. Friedmann[4] recommended reducing the sample to shavings, introducing 2 to 5 g. into a U-tube immersed in a bath at 75° C., drawing a stream of carbon dioxide or nitrogen through the U-tube into either 23 per cent. potassium hydroxide solution or water, and determining the absorbed acetone by Messinger's method[5] by conversion to iodoform with excess of iodine in presence of sodium hydroxide and back-titration with sodium thiosulphate. Although this method has useful features, no evidence is adduced to prove that heating at 75° C. removes the whole of the acetone from the sample, a point which is emphasised by Kostevitch[6] in a criticism of the method used by Benesch,[7] and later by Graulich,[8] of heating samples of propellants to release the volatile matter for estimations. It is well known that solvent matter is tenaciously held by nitrocellulose and its quantitative removal by heat alone is only conceivable with ground material or very small grain or flake samples, and the reduction of larger sizes of cordite to such a state of subdivision introduces the difficulty of avoiding loss of solvent during the process.

These methods were not considered reliable either for routine analysis or for special investigations, and an improved technique was sought. Two possibilities were considered for the quantitative removal of the acetone from a propellant; first, dissolution of the cordite in a suitable medium followed by volatilisation and absorption, and secondly, extraction of the acetone from the cordite by an organic solvent. Since the amount of acetone present in cordites rarely exceeds 0·6 per cent. and also a method suitable for 2- to 3-g. samples was considered necessary, the quantity of acetone to be estimated was of the order of a few mg. Methods applicable to the estimation of small quantities of acetone include Messinger's method,[5] which has been investigated in detail by Goodwin[9]; the hydroxylamine hydrochloride method,[10] in which the hydrochloric acid liberated by the formation of acetone oxime is titrated with standard alkali solution; the formation of double salts of acetone with mercuric salts first employed by Dénigès[11]; colorimetric determination based on the condensation of ace tone with such compounds as *o*-nitrobenzaldehyde[12,13]; and a micro-diffusion method based on the bisulphite reaction described by Winnick.[14] A very useful critical review of methods of estimating small quantities of acetone in air has been given by Komar,[15] who preferred a modification of Messinger's method. An examination of all the above methods indicated that the first two were the most satisfactory. Further, for a given amount of acetone, the equivalent volume of standard iodine solution required in Messinger's method is nearly four times the volume of standard alkali solution of the same normality required in the hydroxylamine hydrochloride method, and this fact was considered to justify a preference for the former method, which later became a necessity on grounds of compatibility of reagents with other constituents of the propellant present during the estimation. With this method of estimating the recovered acetone, the two procedures outlined above for the quantitative removal of the acetone from cordite were investigated.

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* For Parts 1 and 2, see ANALYST, 1946, 71, 483; 1947, 72, 47.

THE QUANTITATIVE REMOVAL OF ACETONE FROM CORDITE BY ENTRAINMENT WITH AIR AND ABSORPTION

The removal of the residual organic solvent matter in a propellant by passing a stream of air through a solution of the sample in nitrobenzene at 100° C. has already been described in the method of estimating ethyl alcohol and ether in nitrocellulose powders, in Part 2 of this series.[16] Cordites dissolve even more readily than nitrocellulose powders in nitrobenzene and this method was therefore adopted for cordites. To absorb the acetone carried over by the air stream, various solvents were investigated; it was found that absorption was incomplete in saturated sodium bisulphite solution, water and dilute sulphuric acid, and concentrated sodium hydroxide solution was not wholly satisfactory. Complete absorption was effected, however, in a 1 : 1 by volume mixture of concentrated sulphuric acid and water. An apparatus was used identical with that described in the method for nitrocellulose powders (see Part 2) except that only one absorption tube was employed in place of the three required in that method. The procedure used in carrying out the separation of the acetone in this way and its subsequent titration was as follows.

ABSORPTION METHOD

About 10 ml. of the sulphuric acid was introduced into the absorption tube, while 2 g. of the cordite sample, cut up into ¼-in. lengths, were added to about 25 ml. of pure nitrobenzene in the 250-ml. three-necked flask. The flask was heated in a boiling water bath and a stream of air bubbled through the nitrobenzene solution to carry the acetone vapour into the sulphuric acid in the absorption tube. After 5 hours, the flow of air was stopped and the sulphuric acid run out and carefully neutralised with 30 per cent. sodium hydroxide solution; 1 drop of phenolphthalein was used as indicator and the solution was cooled during neutralisation. Forty ml. of *N* sodium hydroxide solution were added and, after cooling in ice for 10 minutes, 10 ml. of 0·1 *N* iodine were slowly run in, the flask being shaken during the addition. After the flask had been replaced in ice for 10 minutes, the iodine that had not reacted was liberated by addition of a slight excess of 2 *N* sulphuric acid and titrated with 0·05 *N* sodium thiosulphate, with starch solution as indicator. A blank determination was carried out on 10 ml. of the original diluted sulphuric acid (1 + 1) and the amount of acetone present in the sample calculated. One ml. of 0·1 *N* iodine ≡ 0·0009675 g. of acetone.

The accuracy of the method was tested by introducing a solution of acetone in nitrobenzene of known concentration into the flask, adding the other ordinary constituents of cordite, *viz.*, nitrocellulose, nitroglycerine, nitroguanidine and diethyldiphenylurea, and carrying out an estimation as described above. No interference by these constituents was observed and the results obtained for various solutions of acetone in nitrobenzene given in Table I indicate an accuracy of 3 to 4 per cent. with a maximum error of 5 per cent. for quantities of acetone of the order of 1·5 to 5 mg. No difficulties were encountered in applying the method to the analysis of cordites. Confirmation of the reliability of the method was obtained by applying it to a sample of solventless cordite; no acetone or other organic solvent is used in the manufacture of this type of cordite and an analysis for its acetone content gave a figure of 0·00 per cent. The only unsatisfactory feature of this method is that it is impracticable for dealing with the large numbers of samples encountered in routine analysis. For this reason the possibility of quantitative extraction of the acetone with an organic solvent was investigated.

TABLE I

ABSORPTION METHOD—WEIGHED AMOUNTS OF ACETONE IN NITROBENZENE SOLUTION REMOVED AT 100° C. AND ABSORBED IN SULPHURIC ACID

Weight of acetone taken mg.	Weight of acetone found mg.	Error per cent.
5·31	5·08	−4·3
2·85	2·95	+3·5
2·62	2·49	−5·0
1·88	1·89	+0·5
1·45	1·50	+3·0

THE QUANTITATIVE EXTRACTION OF ACETONE FROM CORDITE WITH AN ORGANIC SOLVENT

The quantitative extraction of moisture from cordite by means of dioxan has already been described in Part 1 of this series.[17] Of possible solvents for the present purpose, dioxan was unsuitable in that it consumed appreciable traces of iodine, and methyl alcohol had to be rejected owing to its dissolving some nitroglycerine, which by its interaction with sodium hydroxide gave rise to decomposition products interfering in the iodine titration. In point of fact, no single organic solvent was found to be suitable for the quantitative extraction of acetone from cordite; eventually, it was found that a mixed solvent consisting of 3 volumes of nitrobenzene and 2 volumes of chloroform satisfied requirements. Trials were made with solutions of acetone of known strength in this mixed solvent, the estimations being carried out directly by Messinger's method; the acetone solution was vigorously stirred with the aqueous sodium hydroxide and standard iodine solution during the time allowed for the acetone and iodine to react. All solutions except the iodine solution were maintained at 0° C. before being mixed in the reaction vessel, which was kept during the estimation in a Dewar flask containing ice. The measured volume of iodine was added slowly from a burette over a period of 5 minutes. In a series of determinations the factors that were varied were the relative volumes of the mixed organic solvent and the aqueous solution, the strength of the sodium hydroxide solution and the length of time allowed for reaction between the iodine and acetone. From the results given in Table·II it can be concluded that (*a*) an increase in the volume of organic solvent relative to the volume of aqueous solution necessitates a longer period of time for completion of the reaction, (*b*) a decrease in the strength of sodium hydroxide solution must be compensated by an increase in the time allowed for reaction, (*c*) quantities of acetone of the order of 5 mg. dissolved in this mixed solvent can be estimated with an accuracy of 2 to 3 per cent. under the optimum conditions. It was ascertained that neither nitrocellulose nor diethyldiphenylurea interfered in the estimation and nitroguanidine is practically insoluble in nitrobenzene and chloroform. A further series of determinations were carried out with nitroglycerine added to the synthetic solutions of acetone. The variation of factors influencing the estimation was modified in relation to the knowledge gained from the first series of determinations and from the results given in Table III it is evident that the extent of interference by nitroglycerine increases with an increase in either the amount of nitroglycerine present or the strength of the sodium hydroxide solution or

TABLE II

ESTIMATION OF ACETONE IN NITROBENZENE - CHLOROFORM SOLUTION

Weight of acetone taken mg.	Volume of organic solvent ml.	Volume of water ml.	Volume of *N* NaOH ml.	Time of reaction min.	Weight of acetone found mg.	Error per cent.
6·36	20	25	15	20	5·05	−20
6·36	20	25	15	40	6·19	−3·0
6·36	20	25	15	40	6·19	−3·0
3·34	15	25	15	20	3·23	−3·3
5·30	15	25	15	10	5·10	−3·9
4·16	15	25	15	20	4·26	+2·4
6·36	15	25	15	25	6·31	−0·9
6·81	15	25	15	25	6·63	−2·9
4·24	15	25	15	25	4·11	−3·1
7·42	15	25	15	30	7·15	−3·6
5·30	15	25	15	35	5·30	0
5·40	10	50	15	15	5·20	−3·7
5·40	10	50	15	20	5·22	−3·3
5·40	10	50	15	27	5·26	−2·6
5·40	10	50	15	30	5·24	−3·0
5·40	10	50	10	15	5·07	−6·1
5·40	10	50	10	20	5·26	−2·6
4·32	10	50	10	20	4·28	−0·9
7·56	10	50	10	20	7·35	−2·8
5·40	10	50	10	25	5·27	−2·4
5·40	10	50	10	25	5·27	−2·4
5·40	10	50	10	25	5·28	−2·2
4·32	10	50	10	25	4·21	−2·5
7·56	10	50	10	30	7·41	−2·0
7·56	10	50	10	40	7·49	−0·9

the length of time allowed for the estimation. As the conditions are approached under which no interference at the end-point occurs, the interval of time between the stage at which the end-point is reached and the return of the blue colour of the starch - iodine complex increases until it is of the same order as that occurring when no nitroglycerine is present, *i.e.*, when the gradual reappearance of the blue colour can be attributed solely to the effect of atmospheric oxygen. The last two results in Table III indicate the optimum conditions; full experimental details based on these conditions are given below. Using this procedure, a sample of solventless cordite which contained no acetone was analysed. This type of cordite has a higher nitroglycerine content than normal cordites, and the amount of nitroglycerine extracted from it by the nitrobenzene - chloroform solvent was obviously much higher than that extracted from ordinary cordites. On two separate samples of this cordite, analyses showed apparent acetone contents of 0·005 and 0·004 per cent. which are negligible. No difficulty was experienced with the end-point and it is clear therefore that the method is quite satisfactory in presence of nitroglycerine. In Table IV is given a comparison of results obtained on some cordites by this method and by the method of entrainment by air from solution in nitrobenzene at 100° C. described above; excellent agreement is evident.

TABLE III

EFFECT OF NITROGLYCERINE ON METHOD OF TABLE II

Volume of nitroglycerine added ml.	Weight of acetone taken mg.	Volume of organic solvent ml.	Volume of water ml.	Volume of *N* NaOH ml.	Time of reaction min.	Weight of acetone found mg.	Error per cent.
0·30	5·84	15	50	10	20	5·22	−11·0
0·10	5·84	15	25	15	20	5·35	−9·0
0·10	5·84	15	50	10	40	5·55	−5·0
0·10	5·84	10	50	10	25	5·70*	−2·4
0·15	3·34	10	50	10	20	3·25*	−2·7

* No interference at end-point of titration.

TABLE IV

DETERMINATION OF ACETONE IN CORDITE—COMPARISON OF EXTRACTION AND ABSORPTION METHODS

Sample	Acetone per cent.	
	Absorption method	Extraction method
Cordite W 124, lot BS 87	0·09; 0·09	0·10; 0·10
Cordite WM 130, lot WAC 1724 ..	0·33; 0·31	0·34; 0·33
Cordite MD St., lot WA 791 ..	0·32; 0·30	0·30; 0·29

Final confirmation of the accuracy and reliability of the method was provided by the following experiment. Three g. of a sample of a W.M. cordite containing mineral jelly and diethyldiphenylurea was allowed to stand in contact with 20 ml. of the nitrobenzene - chloroform solvent overnight. Exactly 5 ml. of the supernatant solvent was then removed and replaced by 5 ml. of a synthetic solution of acetone in the same solvent, and this was again allowed to stand overnight. The acetone contents of both this final solution and of the 5 ml. aliquot portion originally removed were estimated. The estimation of the acetone content of the 5 ml. aliquot portion enabled the acetone content of the residual 15 ml. to be calculated; to this figure was added the known amount of acetone present in the added 5 ml. of synthetic solution, and this was compared with the result of the direct analysis of the final solution. As the results of the duplicate determination given in Table V show, these figures differ by about 2 per cent. This experiment establishes that complete extraction of the acetone occurs on standing overnight, for no increase in the acetone content of the supernatant solvent is detected after allowing to stand for a second time overnight.

For most purposes this procedure of allowing the sample of propellant to stand in contact with the solvent overnight was convenient, but if results are required more quickly, the time of standing can be reduced to a few hours by increasing the amount of nitrobenzene relative to the chloroform in the mixed solvent. When this modification was used, however, it was found that for each particular type of cordite the altered conditions of the estimation varied slightly. Therefore, before this more rapid method is employed, it is necessary to investigate the optimum ratio of nitrobenzene and chloroform and the time to be allowed for extraction for the type of cordite under analysis.

TABLE V

RECOVERY OF ACETONE ADDED TO AN EXTRACTED CORDITE SAMPLE
3 g. of sample in 20 ml. of nitrobenzene - chloroform solvent

		(*a*) mg.	(*b*) mg.
(1)	Weight of acetone found in 5 ml. of supernatant solvent	2·31	2·48
(2)	Weight of acetone in residual 15 ml. of solvent from (1)	6·93	7·44
(3)	Weight of acetone added to residual 15 ml. of solvent	5·40	5·40
(4)	Total weight of acetone present in final solution, *i.e.*, (2) + (3)	12·33	12·84
(5)	Total weight of acetone found in final solution ..	12·6	12·6
	Error	+2·1%	−1·9%

(*a*) and (*b*) are duplicate estimations.

The method is applicable in the presence of ethyl alcohol and has been used without modification for the estimation of acetone in cordites manufactured with a mixed ethyl alcohol - acetone solvent. Details of the method are given below.

EXTRACTION METHOD

Make up the solvent as required by mixing 3 volumes of AnalaR nitrobenzene and 2 volumes of B.P. chloroform. Cut up the sample of cordite into ¼-in. lengths, and add a weighed 3-g. portion to 20 ml. of the solvent in a 25-ml. cylinder fitted with a ground-glass stopper. Stopper the cylinder and allow to stand overnight. After shaking and allowing to settle, transfer 5 ml. of the supernatant solution to a 200-ml. pear-shaped flask and add 5 ml. of the nitrobenzene - chloroform solvent and 50 ml. of water. After stoppering, place the flask in an ice bath, in which is also maintained a *N*-solution of sodium hydroxide. After 15 minutes in the ice bath remove the flask to a Dewar flask containing ice and clamp it vertically with the lower portion immersed in the ice. Remove the stopper and add 10 ml. of the *N* sodium hydroxide. Introduce a motor driven stirrer into the flask and add 10 ml. of 0·1 *N* iodine dropwise over a period of 5 minutes from a 10-ml. burette, keeping the solution efficiently stirred during the addition. Continue the stirring for a further 20 minutes and then remove the flask and add a slight excess of *N* sulphuric acid immediately. Titrate the liberated iodine without delay, with 0·05 *N* sodium thiosulphate. Near the end-point vigorous shaking is necessary to remove the last traces of iodine dissolved in the nitrobenzene - chloroform layer to the aqueous layer. Carry out a blank determination on 10 ml. of the nitrobenzene - chloroform solvent and, after correcting for the very slight consumption of iodine in this "blank" (usually equivalent to 0·05 or 0·10 mg. of acetone per 10 ml. of solvent), calculate the amount of acetone present from the relationship between the iodine solution and acetone given above (p. 435).

SUMMARY

Existing methods of estimating the residual acetone content of cordite are critically reviewed, particularly from the viewpoint of ensuring quantitative removal of the acetone from the cordite prior to its estimation. A new method is described in which the acetone is extracted with a mixed nitrobenzene - chloroform solvent and estimated by Messinger's method with an accuracy of 2 to 3 per cent. Interference by nitroglycerine has been studied in detail and the conditions established for its complete elimination.

In conclusion, I should like to thank Mr. G. L. Hutchison, of the Armament Research Department for his helpful advice, and the Director-General of Scientific Research (Defence), Ministry of Supply, for permission to publish this material.

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Spot-tests for the Detection of Alloying Elements in Tin-Base Alloys[*]

BY B. S. EVANS AND D. G. HIGGS

TIN is extensively used as the basis material for the manufacture of bearing metals and solders. The composition of bearing metals varies considerably according to the particular work required of them. The alloying elements are usually some or all of the following: lead, antimony, zinc, copper and aluminium in varying proportions. Tests have been worked out for lead, copper, arsenic, antimony, zinc and aluminium.

Apparatus—In addition to the droppers, stirrers and capillary tubes already described in former papers[4] the following are needed: (*a*) white porcelain spot-plate, and (*b*) apparatus used for the detection of arsenic (*vidè* (III) Arsenic, and this Vol., p. 108).

Cleaning the specimen—The surface of the sample is thoroughly cleaned by rubbing it with emery paper—Grade "F" (Buff), or similar—prior to addition of the attacking reagent.

The tests—Of the tests described, those for lead, copper and arsenic are completed on the surface of the sample; the tests for antimony, zinc and aluminium require for completion the removal of the reaction drops from the specimen at some intermediate stage. The test described for lead is the only one of this series that is new to spot-testing. The tests for the remaining elements are either identical with or modifications of tests published earlier, *e.g.*, antimony and arsenic: "Lead-base Alloys"[1]; aluminium: "Steels"[2] and "Zinc-base Alloys"[3]; copper and zinc: "Aluminium- and Magnesium-base Alloys."[4] Iron is seldom added to tin-base alloys, but is usually found as an impurity varying in amounts from 0·02 to 0·05 per cent. The only specification[5] in which iron is a constituent gives a figure of 0·10 per cent. Fe, and since no spot-test could be expected to differentiate between an alloying content of 0·1 per cent. and an impurity of 0·02 to 0·05 per cent., a test for it has not been included.

(I) LEAD

Reagents—(*a*) Nitric acid (sp.gr. 1·42).
(*b*) Distilled water.
(*c*) Urea (solid).
(*d*) Potassium iodide solution (4 per cent.).

Method—Place 2 drops of (*a*) on the thoroughly cleaned surface and leave them to react until a solid crust has formed or, for less reactive alloys, until the reaction appears to be complete. Add 2 drops of (*b*) and stir; then add a small quantity of (*c*), sufficient to form a small heap on the end of a pen-knife blade and again stir until the crystals are well mixed with the drop. Add 2 or 3 drops of (*d*) and stir thoroughly. In presence of much lead, say above 10 per cent., a very heavy bright yellow precipitate, and down to about 1 per cent. a heavy yellow precipitate, is formed; with about 0·5 per cent. of lead a slight yellow precipitate only is produced. In absence of lead the composite drop on the specimen is water-white. The precipitate of lead iodide may be transferred to a filter-paper, dried and retained for future reference.

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[*] Communication from the Armament Research Department (formerly the Research Department, Woolwich).

Tried on: Samples Nos. 10, 7, 12, 11, 22, 15, 8, 6, 4, 3, 14, 5, 9, 13, 1, 23, 21—Lead present and all gave positive results.
Nos. 2, 16, 24, 25, 26—Lead absent and all results negative.

(II) COPPER

Reagents—(*a*) Nitric acid (sp.gr. 1·42).
(*b*) Diluted ammonia (1 + 1).
(*c*) Mixture of α-benzoin-monoxime (saturated solution in alcohol), 10 vols.; diluted ammonia (1 + 1), 20 vols.; citric acid solution (50 per cent.), 5 vols.

Method—Place 2 drops of (*a*) on the thoroughly cleaned surface and leave to react until the attack is complete, add 2 drops of (*b*), stir well (the drop should now be alkaline), then add 4 to 6 drops of (*c*), again stir well and leave for 5 to 10 minutes. In presence of copper a dirty green complex separates out slowly, whilst in absence of copper the drop remains water-white. The deep blue coloration produced on addition of the 2 drops of (*b*) when copper is present is quite unmistakable and could be used as a quick test for copper.

Tried on: Samples Nos. 6, 21, 22, 1, 16, 5, 13—Copper present and all results positive.
Nos. 14, 15, 2, 3, 4, 7, 8, 9, 10, 11, 12, 24, 25—Copper below 0·05 per cent. and all results negative.

(III) ARSENIC

Reagents—(*a*) Diluted hydrochloric acid (1 + 1) saturated with bromine.
(*b*) Hydrochloric acid (concentrated).
(*c*) Arsenic-free zinc.
(*d*) Mercuric chloride test paper.
(*e*) Ferrous sulphate (small crystals).

Apparatus—(*g*) A glass tube 1 in. by ⅝ in., ground flat at both ends, (*h*) a glass tube 2¾ in. by ⅜ in. fitted at one end with a half-inch length of rubber tubing so arranged that a little of the tubing overlaps the end of the glass tube, forming a slightly tapered end which serves as a stopper; this tube with its rubber end should fit snugly into tube (*g*), (*j*) rubberised vaseline such as is used for stop-cock lubricants, and (*k*) cotton-wool.

To prepare the tube (*h*) for the test, insert a tightly packed plug of cotton-wool into the tube so that its lower end is about ½ in. from the bottom of the tube, the plug being about ½ in. deep. To the rubber-tubing end of the cotton-wool plug add fine crystals of (*e*) until a layer about ¼-in. thick has been formed, insert a second plug of cotton-wool to prevent the ferrous sulphate from falling out when the tube is in position for the test. Cut a strip of paper (*d*) about ¼ in. by 1¼ in., insert it in the glass tube and then plug the free end of the tube loosely with cotton-wool; the tube is now ready for the test.

Method—Thoroughly clean the surface of the specimen with emery paper. Dip the end of the glass tube (*g*) into the hot sealing grease (*j*), then withdraw it and, after making sure that there is no film of grease across the end, press it firmly on the specimen and allow the grease to set. Add 4 drops of (*a*) inside the wide tube and leave to react for 5 minutes. Add 15 drops of (*b*). Drop a small granule of (*c*) into the wide tube and allow the reaction to proceed for a few seconds; then, before the bromine has been destroyed, insert the rubber-stoppered end of the prepared tube (*h*) and leave for 15 minutes. Detach the top tube, (*h*), remove the top plug and shake out the test strip. A yellowish-brown stain on the strip indicates the presence of arsenic. Four samples gave a slight yellow coloration of the bottom ⅛ in. of the strip, and subsequent chemical analysis has shown that these samples contained 0·014, 0·011, 0·020 and 0·027 per cent. of arsenic respectively. In absence of arsenic the paper remains quite white; this is in sharp contrast to the same test as applied to lead-base alloys, where heavy greyish-black stains were produced by high antimony contents.

Tried on: Samples Nos. 13, 2, 3, 4, 14—Arsenic present and all results positive.
Nos. 1, 5, 6, 7, 8, 9, 10, 11, 12, 15, 16, 21, 22, 23, 24, 25—Arsenic absent and all results negative.

(IV) ANTIMONY

Reagents—(*a*) Nitric acid (sp.gr. 1·42).
(*b*) Hydrochloric acid (concentrated).
(*c*) Potassium nitrite (solid).
(*d*) Rhodamine "B"[6] (0·01 per cent. solution in water).

Method—Place 2 drops of (*a*) on the thoroughly cleaned surface and leave to react until a solid crust has formed, or, for the less reactive alloys, until the reaction appears to be complete. Add 3 drops of (*b*) and transfer quickly, by means of a capillary tube, to a well of a white porcelain spot plate. Add a further 2 drops of (*b*) and then a few small crystals of (*c*) and leave to react for about half a minute. In another well of the spot-plate place 4 drops of (*d*) and add to them 2 or 3 drops of the solution from the first well; stir, add 2 more drops of (*b*), stir again, then leave. In presence of much antimony an immediate heavy purple precipitate or coloration is produced; 0·12 per cent. of antimony gives a purplish coloration to the drop. In absence of antimony the reagent drop has a dirty reddish-pink colour. A positive reaction was given by three samples to which antimony had not originally been added, but chemical analysis has shown that the samples contained 0·26, 0·22 and 0·15 per cent. respectively. While removing the reaction solutions to filter-paper for future reference it was noticed that samples that gave a dirty reddish-pink colour on the spot-plate (regarded as having no antimony present) now gave slight purplish precipitates when washed once or twice with water. All samples not supposed to contain antimony were tested on filter-paper and it was found that down to about 0·06 per cent. of antimony could be detected in this way. AnalaR tin, spectrographic tin and the reagents alone did not give a purple precipitate. When dry, the purplish precipitates became mauvish in colour. It is recommended that removal of the drop to a filter-disc should not be used as part of the test.

Tried on: Samples Nos. 15, 21, 1, 11, 6, 22, 10, 7, 12, 13, 4, 2, 5, 16—Antimony present and all results positive.
Nos. 3, 9, 14, 24, 8, 26, 27—Antimony below 0·09 per cent. and all results negative.

The antimony in samples Nos. 3, 9, 14, 24, and 8 was first detected by removal of the reaction drop to a filter-disc and subsequently confirmed by chemical analysis of another portion of each alloy.

(V) ZINC

Reagents—(*a*) Nitric acid (sp.gr. 1·42).
(*b*) Urea (solid).
(*c*) Diluted ammonia (1 + 1), 1 vol.; ammonium acetate* solution, 2 vols.; potassium cobalticyanide solution (10 per cent.), 2 vols.
(*d*) Diphenylcarbazone (1·5 per cent. solution in alcohol).
(*e*) Acetone.
(*f*) *Iso*propyl alcohol.

Method—Place 1 drop of (*a*) on the thoroughly cleaned surface and leave until the reaction appears to be complete; add a small heap of (*b*), sufficient to cover the point of a pen-knife blade, follow with 5 drops of mixture (*c*) and stir thoroughly. Add 5 drops of (*d*), stir, and after a few seconds transfer, by means of a capillary tube, to the centre of a disc of close-grained filter-paper supported on the open mouth of a beaker; the transfer should be made 3 or 4 drops at a time in order to spread out the spot for subsequent acetone washing. Allow to spread completely, then wash three times with 3 drops of (*e*), and continue with three or four 3-drop washings of (*c*). Zinc, lead and copper all give precipitates under the conditions existing before treatment of the transferred drop; copper slowly destroys the reagent colour, the decolorisation being greatly accelerated by acetone. The acetone wash also dissolves, decolorises and washes out the lead precipitate, whilst the deep blackish-purple precipitate due to zinc is dissolved and spread out over a limited area as a deep permanganate-coloured patch. Washing with *iso*propyl alcohol (*f*) removes any incidental reagent colours but leaves the zinc patch in its original position. In presence of zinc an intense blackish-purple spot at the centre of the paper, surrounded by a permanganate-coloured patch about 1½ to

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* Preparation of ammonium acetate solution: add, to 1140 ml. of distilled water in a 3-litre porcelain beaker placed in a cooling bath, 500 ml. of 0·880 ammonia solution followed by 570 ml. of glacial acetic acid, the solution being stirred continuously. Allow to cool; the solution should be neutral.

2 in. in diameter, is obtained. In absence of zinc the paper has a clear centre (sometimes a slight light brown or pink smudge due to traces of copper or lead precipitates not completely washed out) and a narrow ring about 2½ in. in diameter, which varies in colour from light brown to maroon. Zinc down to 0·47 per cent. gives a fair reaction. Where the presence of zinc is doubtful it is best to wait 5 minutes after the last addition of (*f*) before deciding whether the centre contains a precipitate or a colour which *does not* fade.

Tried on: Samples Nos. 5, 13, 14, 3, 16, 2, 4, 19, 20, 18, 17, 15—Zinc present and all results positive.
Nos. 1, 6, 7, 8, 9, 10, 11, 12, 21, 22, 23, 24, 25—Zinc absent and all results negative.

(VI) ALUMINIUM

Reagents—(*a*) Concentrated hydrochloric acid saturated with bromine.
(*b*) Mixture of sodium hydroxide solution (20 per cent.), 3 vols.; potassium cyanide solution (10 per cent.), 1 vol.
(*c*) Ammonium aurintricarboxylate (0·01 per cent. solution in alcohol).
(*d*) Mixture of ammonium chloride solution (20 per cent.), 1 vol.; ammonium phosphate solution (10 per cent.), 1 vol.
(*e*) Ammonium chloride solution (20 per cent.).

Method—Place 2 drops of (*a*) on the thoroughly cleaned surface and allow them to react until the bromine colour is all but dispelled, then add 4 or 5 drops of (*b*) stir well, and if the heavy white gelatinous precipitate has not dissolved add a further drop of (*b*). Treat a circle of close-grained filter-paper with 1 ml. of reagent (*c*), causing it to spread evenly and finally drying over a source of hot air. Place the paper on the open mouth of a beaker and add the reaction solution, which is removed from the sample by means of a capillary tube, to the centre of the paper, in a dropwise manner. When the last drop has been added and the liquid

TABLE I

SPECIMENS OF ALLOYS USED IN THE TESTS. CONSTITUENTS PER CENT.

No.	Mark	Pb	Sb	As	Cu	Zn	Al	Fe
1	DOF	0·05	9·75	—	3·60	—	—	—
2	EX 14	—	0·22*	0·014*	—	7·60	3·87	—
3	EX 15	3·41	0·09*	0·011*	—	11·41	—	—
4	EX 16	4·38	0·26*	0·020*	—	6·10	—	—
5	EX 7	1·10	0·15*	—	2·67	40·9	—	—
6	EX 46	8·37	7·0	—	9·35	—	—	—
7	EX 47†	39	3	—	—	—	—	—
8	EX 48†	20	0·06*	—	—	—	—	—
9	EX 49†	1·0	0·09*	—	—	—	—	—
10	EX 50	46·95	3·11	—	—	—	—	—
11	EX 51	23·25	9·57	—	—	—	—	—
12	EX 52	38·65	2·24	—	—	—	—	—
13	EX 53	0·84	1·20	0·40	1·63	25·34	0·03	0·10
14	EX 54	1·31	0·09*	0·027*	0·03	19·16	0·26	—
15	EX 55	19·45	11·12	—	0·04	0·36	—	—
16	EX 56	—	0·12	—	3·44	9·27	—	—
17	EX 78	0·56	—	—	—	0·46	—	—
18	EX 79	30·24	—	—	—	0·50	—	—
19	EX 80	0·68	—	—	—	1·33	—	—
20	EX 81	30·63	—	—	—	1·11	—	—
21	BCN	0·37	9·89	—	3·91	—	0·02	—
22	BCM	21·94	5·63	—	3·81	—	0·01	—
23	USA	0·49	—	—	—	—	—	—
24	"Pure" Tin	—	0·08*	—	—	—	—	—
25	"Chempure" Tin	—	—*	—	—	—	—	—
26	"Spectro" Tin	—	—	—	—	—	—	—
27	A/R Tin	0·01	0·03	—	—	—	—	—

* Found first by spot-test and later confirmed by chemical analysis.
† Samples not analysed chemically.

has finished spreading, remove the filter paper from the beaker, place it flat on a clean porcelain tile and cover it with a 7-cm. filter-paper thoroughly wetted with mixture (*d*); press the latter paper on to the reaction filter-paper, to ensure good contact at all points and after a few seconds strip it off and reject it. Return the original paper to its beaker, leave it for 5 minutes, then wash it twice with 3 or 4 drops of (*e*) and finally dry it. In presence of aluminium the paper shows a sharp pinkish-crimson irregular ring about 1¼ in. in diameter outside which is a broad band of colour, similar to that of the ring but less intense; the ground both inside and outside the ring should be white while wet. In absence of aluminium the paper should be completely white after 5 minutes standing. With 0·26 per cent. of aluminium, instead of the characteristic markings of the high aluminium contents there is a single light pinkish irregular band of the same diameter.

Tried on: Samples Nos. 2, 14—Aluminium present and both give positive results.
Nos. 1, 3, 4, 5, 6, 7, 10, 11, 12, 13, 15, 16, 21, 22, 23, 24—Aluminium below 0·05 per cent. and all results negative.

SUMMARY

Tests are described for the detection of alloying elements in tin-base alloys. The alloying elements so detected are lead, copper, arsenic, antimony, zinc, and aluminium. With the alloys available the tests are specific and unambiguous. No test has been included for iron since the margin between impurity and alloying constituents is too small.

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May, 1947

A System of Qualitative Analysis for the Common Metals in Presence of Phosphates, using Ammonium Benzoate

BY G. J. AUSTIN

THE benzoate iron group separation[1] has been proposed[2,3] for group analysis, instead of the more common separation by ammonia. It gives much better separations than other methods and can be used in presence of phosphate. However, some of the phosphate passes into the filtrate and when ammonia is added phosphates of the bivalent metals may precipitate, thus interfering with the rest of the analysis. This is overcome in the system now proposed by using some of the principles employed in my phosphate method[4,5] in which the solution remains acid until all the metals except magnesium and alkali metals have been separated.

This system of analysis can be applied regardless of the presence of phosphate and, except when 0·20 g. or more of phosphate (P_2O_5) is present, it will usually give much better separations than my phosphate or other methods. However, when phosphate is present the iron group precipitate is unsuitable for direct ignition and weighing, as a mixture of oxides and phosphates is obtained. The phosphate method[4,5] can be used in such instances.

An important advantage of the benzoate method of separating iron group metals, which is used in this system, is that it can be used when chromium is the only iron group metal present. Under these conditions chromium cannot be precipitated as basic acetate, formate or succinate. Uranium, beryllium, and tervalent cerium are only partially precipitated in the benzoate separation unless sufficient phosphate is present to precipitate these metals as phosphates, but these metals and also chromium can be precipitated in my phosphate method.

When this system is used for ordinary qualitative analysis it is sufficient to wash the precipitates with water. Until the 5 g. of ammonium acetate is added, the solution is apt to deposit benzoic acid on cooling, but this will redissolve on warming.

A SYSTEM OF ANALYSIS SUITABLE FOR PHOSPHATES

Remove Groups I and II as usual. (Chromates and $Fe^{\cdots}$ are best reduced with HI—see ref. 4.) To a solution of 0·5 g. in 20 ml. add dilute ammonia until a slight ppt. persists. Add 1 ml. of 5 *N* HCl. Heat to incipient boiling, add 2 ml. of 10% $(NH_4)_2SO_4$ solution and if there is a ppt. add another 2 ml. of $(NH_4)_2SO_4$ solution and heat in boiling water for 15 to 30 min. Filter and wash with 0·05 *N* H_2SO_4.

<table>
<tr><td rowspan="6"><i>Ppt.</i>
$SrSO_4$
$BaSO_4$
Try flame test and/or boil with 50 ml. of <i>N</i> Na_2CO_3. Decant through filter. Repeat process. Dissolve the carbonates in acetic acid. Add K_2CrO_4: ppt. = $BaCrO_4$. To solution add H_2SO_4: ppt. = $SrSO_4$.</td><td colspan="6"><i>Solution.</i> Add sat'd. bromine solution and boil off excess. To the cold solution add dilute ammonia until a slight ppt. persists. Add 1 ml. of glacial acetic acid and 2 g. of NH_4Cl, dilute to 100 to 150 ml. Heat and add 20 ml. of 10% ammonium benzoate solution. Boil. (<i>p</i>H is now 3·8 to 4·0.) Filter hot and wash with hot benzoate wash solution or with 2% ammonium nitrate solution.</td></tr>
<tr><td rowspan="5"><i>Ppt.</i>
Fe, Al, and Cr as phosphates and/or basic benzoates
Suspend in water, add H_2O_2 and NaOH solution. Boil, dilute and filter; $Fe(OH)_3$ remains. To solution add ammonium phosphate solution and excess of acetic acid; $AlPO_4$ is pptd. If the solution is yellow, Cr is present. To confirm Cr add diphenyl carbazide—gives red colour.</td><td colspan="5"><i>Solution.</i> Add 5 g. of ammonium acetate. (<i>p</i>H is now 4·6 to 5·0.) Heat to boiling and pass H_2S through the hot solution for 5 to 10 min. Boil for a few seconds and filter. Wash with 0·1 <i>N</i> acetic acid.</td></tr>
<tr><td rowspan="4"><i>Ppt.</i>
ZnS, CoS, NiS.
If Zn is present white ZnS pptes. before the black CoS or NiS. Dissolve the sulphides in 1:1 HNO_3 and test parts for Ni, Co and Zn.</td><td colspan="4"><i>Solution.</i> Boil off H_2S. To the gently boiling solution add 5-ml. portions of the periodate reagent until a positive spot test for periodate with the starch iodide solution is obtained. Filter and wash with a little water and then with hot 0·1 <i>N</i> HNO_3.</td></tr>
<tr><td rowspan="3"><i>Ppt.</i>
$MnO_2.H_2O$.
To confirm Mn, extract the ppt. with KHC_2O_4 solution; a deep red colour proves Mn.</td><td colspan="3"><i>Solution.</i> To the hot solution add 5 ml. of <i>M</i> citric acid and 25 ml. of sat'd. ammonium oxalate solution. Allow to stand at least 5 min., filter and wash.</td></tr>
<tr><td rowspan="2"><i>Ppt.</i>
$CaC_2O_4.H_2O$.</td><td colspan="2"><i>Solution.</i> Concentrate to about 150 ml. Add 5 ml. of <i>M</i> ammonium phosphate and to the hot solution add 20 ml. of conc. NH_3. Stand overnight. Filter and wash with 2% NH_3.</td></tr>
<tr><td><i>Ppt.</i>
$MgNH_4PO_4.6H_2O$</td><td><i>Solution.</i>
Reject.</td></tr>
</table>

The 20 ml. of ammonium benzoate solution are sufficient for 0·10 g. of aluminium or 0·20 g. of iron or chromium. For larger amounts of these metals, add the ammonium benzoate solution until the solution has *p*H 3·5 to 4 as indicated by a blue spot test with bromophenol blue indicator. At *p*H 3·0, after 5 minutes boiling, 0·2 mg. of iron or aluminium is left in solution, while at *p*H 3·5 to 4·0 less than 0·1 mg. remains dissolved. When present alone, chromium requires longer boiling than iron or aluminium; after 20 minutes boiling 3 mg. of chromium remained dissolved at *p*H 3·0 and 1 mg. at *p*H 3·5. However, when iron or aluminium was also present then usually not more than 0·1 mg. of chromium remained dissolved after 5 minutes boiling at *p*H 3·5 to 4·0. For ordinary qualitative analysis it is usually sufficient to boil for about one minute before filtering off the basic benzoates.

Table I compares adsorption or co-precipitation of bivalent metals by precipitates of the iron group obtained by various methods. The tests by the ammonia method[3] were with phosphate absent and at *p*H 6·7 to 7·3. The tests by my phosphate method were with 0·355 g. of P_2O_5 present. It is evident that as a general method the benzoate method is best. It may be noted that by ignoring any phosphate present and using the benzoate method as in this system of analysis, the adsorption of zinc, nickel, and cobalt is considerably less than if the phosphate is first removed and then the ammonia method used.

Adsorption losses in the system now described, other than in the iron group, are very small; some examples are given in Table II.

TABLE I

ADSORPTION OF BIVALENT METALS IN THE IRON GROUP

The figures represent the amounts of the various bivalent metals co-precipitated, expressed as percentages of the amounts taken

Weight of metals taken		Adsorption by benzoate method			Adsorption by phosphate method[4]	Adsorption by ammonia method[3]	Adsorption by acetate method[4]
		phosphate absent	0·072 g. P_2O_5 present	0·21 g. P_2O_5 present			
g.	g.	%	%	%	%	%	%
0·055 Al	0·127 Zn	1·4	4·9*a*	14	8	19	15
„	0·116 Mn	0·20	0·8	2·4	1·2	0·10	0·5
„	0·120 Co	0·33	0·8	1·5	1·0	6·2	—
„	0·120 Ni	0·8	2·2	0·8	0·5	71	1·3
„	0·097 Ca	< 0·2	< 0·2	0·6	< 0·2	—	—
„	0·069 Mg	< 0·2	< 0·2	< 0·2	< 0·2	—	—
0·093 Fe	0·127 Zn	0·2	3·0*b*	11	10	12	13·5
„	0·116 Mn	0·1	0·35	3·2	3·0	2·7	4·5
„	0·120 Co	0·1	0·8	2·6	0·7	6·0	4·0
„	0·120 Ni	0·1	0·5	1·7	0·3	6·8	6·0
„	0·097 Ca	< 0·2	< 0·2	0·6	< 0·2	—	—
„	0·069 Mg	< 0·2	< 0·2	< 0·2	< 0·2	—	—
„	0·171 Ba	< 0·1*	< 0·1**c*	30*	30*	0·2	14
0·088 Cr	0·127 Zn	4·5	15	29	26	90	—
„	0·116 Mn	0·4	3·8	10	8	3·2	—
„	0·120 Co	0·8	4·5	4·5	5·5	75	—
„	0·120 Ni	1·6	4·3*d*	4·5	3·5	90	—
„	0·097 Ca	0·2	0·7	7·0	5·0	0·8	—
„	0·069 Mg	< 0·2	0·5	1·5	1·5	1·5	—
„	0·171 Ba	< 0·1*	6·0*	37*	52*	0·8	—
0·022 Al 0·037 Fe 0·035 Cr	0·080 Zn	1·5	2·9	25	14	33	20
0·046 Fe 0·044 Cr	0·116 Mn	0·3	1·0	5·6	7	—	—
0·028 Al 0·044 Cr	0·116 Mn	0·20	1·0	5·8	3·4	—	—

* The adsorption of barium and strontium is avoided by prior removal of these metals as sulphates

a 52% of the P_2O_5 taken was in the filtrate.
b 8% „ „ „ „ „
c 2·3% „ „ „ „ „
d 30% „ „ „ „ „

TABLE II

TESTS ON ADSORPTION ELSEWHERE THAN IN THE IRON GROUP

Taken g.	
Zn ≡ 0·2000 of ZnS Mn ≡ 0·2000 of MnS 0·142 of P_2O_5	The ZnS, weighed as sulphate, gave Zn ≡ 0·2012 g. of ZnS and contained 0·55% of the Mn taken. The MnO_2 was ignited at 1000° C. and weighed as Mn_3O_4. Obtained Mn ≡ 0·1992 g. of MnS.
Ni ≡ 0·200 of NiS Mn ≡ 0·200 of MnS 0·142 of P_2O_5	Less than 0·1% of the Mn taken was present in the nickel sulphide.
Co ≡ 0·200 of CoS Mn ≡ 0·200 of MnS 0·142 of P_2O_5	Less than 0·1% of the Mn taken was present in the cobalt sulphide.
Ca ≡ 0·1500 of CaO Mg ≡ 0·1500 of MgO 0·142 of P_2O_5	1·3% of the magnesium was carried down by the calcium oxalate. Obtained $CaCO_3$ ≡ 0·1511 g. of CaO. „ $Mg_2P_2O_7$ ≡ 0·1483 g. of MgO.
Fe ≡ 0·1000 of Fe_2O_3	Iron ppt. ignited gave 0·1000 g. of Fe_2O_3
Mn ≡ 0·1000 of MnO	Mn in MnO_2 estimated as $Mn_2P\ O_7$ 0·1005 g. of MnO
Ca ≡ 0·1037 of CaO	Calcium was re-pptd. Ignition gave $CaCO_3$ ≡ 0·1043 g. of CaO
Mg ≡ 0·0963 of MgO	Mg ppt. ignited gave $Mg_2P_2O_7$ ≡ 0·0966 g. of MgO

REAGENTS USED—

Sodium periodate solution—Dissolve 4·0 g. of the salt ($Na_2H_3IO_6$) by warming in about 190 ml. of water containing 1·5 ml. of glacial acetic acid. Dilute to 200 ml. and filter.

Starch iodide—Prepare 1 per cent. starch solution and in 100 ml. dissolve 10 g. of sodium bicarbonate and 1 g. of potassium iodide.

Benzoate wash solution—This contains 1 g. of ammonium benzoate and 2 ml. of glacial acetic acid per 100 ml.

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18 STRATTON DRIVE, BARKING, ESSEX

February, 1947

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Colorimetric Determination of Iron and Manganese in Foods. L. C. E. Kniphorst (*Chem. Weekblad*, 1946, **42**, 311–316, 328–334)—An investigation was made of the determination of iron in foods, after wet combustion, by means of sulphosalicylic acid. In the determination of iron alone, phosphates, up to a proportion of 4000 parts of phosphorus pentoxide to 1 of iron, do not interfere. The sulphosalicylic acid has a solvent action on iron phosphate. Large quantities of ammonium sulphate are without influence on the colour. In presence of calcium and magnesium salts, large quantities of ammonium salts are necessary to prevent the precipitation of calcium phosphate or of magnesium hydroxide. Manganese gives a brownish-yellow colour with the reagent, but does not interfere if the ratio of manganese to iron does not exceed 10 to 1, and the colour is matched within one hour. The method for iron and manganese together is applied to a solution, obtained by wet combustion, containing less than 10 mg. of calcium oxide, 10 mg. of magnesium oxide, 60 mg. of phosphorus pentoxide, and 1 mg. of iron. If the amount of manganese found is more than 0·1 mg., the determination should be repeated with less material.

Procedure—Carry out a wet combustion with sulphuric and nitric acids, completing with hydrogen peroxide in order to obtain a solution as free from colour as possible. Add 10 ml. of water, boil for 3 min., and cool. Add to the liquid a piece of Congo Red paper and a piece of litmus paper, and neutralise with aqueous ammonia so that the Congo Red paper is red and the litmus paper pale red. Then add 5 ml. of a suspension of zinc hydroxide containing 160 mg. of zinc hydroxide. This precipitates the iron. Filter the mixture and wash the precipitate with a small quantity of water. Add to the filtrate 0·1 ml. of copper sulphate solution (containing 0·01 mg. of copper), 0·4 ml. of formaldoxime reagent, and 2 ml. of 25 per cent. aqueous ammonia solution (traces of copper accelerate the colour development). After allowing the solution to stand for one hour, make the volume up to 100 ml., and compare the colour with that of standard manganese solutions. The standard contains 0·1 mg. of manganese, and is prepared with sulphuric acid in the same way as the solution to be tested. A control test, containing 0·1 mg. of iron and 0·1 mg. of manganese, is also prepared. The difference between the control and the standard gives the correction to be applied for manganese in the reagents. Dissolve the zinc hydroxide, containing the iron, in 20 ml. of hot, 4 *N* sulphuric acid, and wash the filter three times with small portions of water. Treat part of the solution, containing about 0·1 mg. of iron, with 2 ml. of 20 per cent. sulphosalicylic acid solution, approximately neutralise with aqueous ammonia, and add a few drops in excess. Sufficient aqueous ammonia must be added to remove any turbidity due to precipitated zinc phosphate. Compare the colour with a standard solution containing 0·1 mg. of iron, and also carry out a blank test on the reagents. Results obtained on a number of foodstuffs are as follows:

Material	Iron	Manganese
	mg. per 100 g.	
Green peas	7·5; 7·6	0·99; 1·18
Household syrup ..	30·5	0·73; 0·80
Cooking syrup ..	26·9	0·94
Potato starch ..	3·5; 3·2	0·53; 0·43
Wheat germ	10·8; 10·5	17·0; 16·9

G. M.

Haemoglobin in Meat Scraps and Tankage. R. Reiser (*Anal. Chem.*, 1947, **19**, 114–118)—According to the definition of the Association of American Feed Control Officials Inc. (Offic. Publ., 1946), meat scrap differs from tankage in that blood is excluded from the former but not from the latter. Presence of more than "such traces of blood as might occur unavoidably in good factory practice" thus constitutes adulteration.

There is evidence also that the biological value of blood meal protein for growth is low and that its biological value, palatability, and digestibility decrease with increasing temperature of preparation (Morris, *et al.*, *J. Dairy Res.*, 1936, **7**, 97; Winter, *Ohio Agr. Expt. Sta. Bull.*, 1929, 436). There is need, therefore, for a method to determine blood meal in meat scraps and tankage and for a means of determining the degree of heat treatment to which a sample of blood meal has been subjected.

A method for the determination of blood in packing-house by-products has been reported (Reiser *et al.*, *Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 851; *cf.* ANALYST, 1942, **67**, 302). The sample, after extraction with ether, was boiled in 1 per cent. sodium hydroxide solution, the mixture was added to pyridine, made up to a definite volume with water, and filtered. An aliquot of the filtrate was reduced with sodium hyposulphite ($Na_2S_2O_4$) and the increase in optical density at 550 mμ. was determined in a spectrophotometer by comparing it with that of the unreduced solution.

It has been found that dried blood dissolves more readily in an alcoholic solvent, that a greater colour intensity per unit of haemoglobin is produced, that no loss of colour occurs during boiling, and that preliminary extraction with ether is then unnecessary. Variations in the optical density of the haemochromogens of blood are so great that the customary standards cannot be used, and it thus became necessary to develop a new method for the determination of the extinction coefficient of the haemoglobin in blood meal.

The effects of conditions such as the alcoholic concentration, alkalinity, pyridine concentration, and particle size of the sample on the intensity and stability of the resulting haemochromogen were studied exhaustively. Alcoholic solutions produced more colour than aqueous solutions with no change in the colour intensity in the alcoholic concentration range of 20 ml. to 60 ml. per 100 ml. of solution. Maximum solubility of the blood meal occurred when the alcoholic concentration was 50, 55, or 60 ml. per 100 ml. Complete solution in 5 per cent. sodium hydroxide was obtained in 10 min. with blood meal and in 20 min. with tankage. Visual observations showed that boiling for 20, 10, and 7 min. was necessary to attain complete solution of blood meal samples ground to pass through 20-, 40-, and 60-mesh sieves, respectively. Samples should therefore be ground to pass a 40-mesh sieve. Pyridine aids solution and should therefore be added as part of the solvent.

To determine the extinction coefficient of haemochromogen in blood meal 19 samples were obtained from 13 sources with, in many instances, details of the drying process used. Iron was determined in each sample by digesting 50 mg. in a 100-ml. flask with 4 ml. of sulphuric acid and completing the digestion with 30 per cent. hydrogen peroxide. About 50 ml. of water and 2 ml. of 10 per cent. iron-free hydroxylamine hydrochloride solution were then added, followed by 1 ml. of 1·5 per cent. alcoholic *o*-phenanthroline solution. Aqueous ammonia was added until the liquid changed the colour of Congo Red paper and the resulting colour was read in a photo-electric colorimeter with a 440 mμ. filter. The iron content multiplied by 100 divided by 0·335, the iron content of haemoglobin, gave the haemoglobin content. The optical density of the haemochromogen of each sample of blood meal was then determined by boiling 100 mg. for 30 min. in 100 ml. of a solution containing 55 ml. of 95 per cent. alcohol, 5 g. of sodium hydroxide, 5 ml. of pyridine, and water to 100 ml. A 10- or 20-ml. aliquot was then reduced with a few mg. of sodium hyposulphite ($Na_2S_2O_4$) and the increase in optical density of the reduced solution over that of the unreduced solution at 553 mμ. was determined. The extinction coefficient of the haemoglobin was then calculated from the haemoglobin content by use of the relation $e = D/CL$, where e is the extinction coefficient, D the optical density of the haemochromogen, C is the percentage of haemoglobin in the solution, and L is the depth of the solution in centimetres. It was found that the extinction coefficient of blood that had been heated for less than 7 hr. in steam-jacketed dryers at less than 55 lb. pressure was 5·4, whereas with blood that had been heated for over 7 hr. at over 55 lb. pressure the corresponding coefficient was 3·6. An investigation of the coefficient of extinction of fresh beef blood that had been submitted to varying degrees of heat for varying periods showed that, according to the heat-treatment applied, the extinction coefficient reached a minimum value of 5·4 and, with more drastic treatment, a minimum value of 3·3, these being in close agreement with the corresponding values found for blood meal.

Procedure—To 1 g. of finely ground meat scraps or 0·5 g. of tankage in a 300-ml., ground-glass-jointed Erlenmeyer flask add 100 ml. of a solution containing 55 ml. of 95 per cent. alcohol, 5 ml. of pyridine, 5 g. of sodium hydroxide, and water to volume. Heat under refluxing conditions for 30 min., cool, and centrifuge. To 10 or 20 ml. of the clear supernatant liquid add a few mg. of sodium hyposulphite ($Na_2S_2O_4$) and determine the increase in the optical density at 553 mμ. of the reduced solution over that of the unreduced solution. Calculate the haemoglobin content from the relation $Hb = 100DV/100eLS$, where Hb is the percentage of haemoglobin in the sample, e is the extinction coefficient, D is the increase of optical density, L is the depth of solution in cm., S is the weight of the sample in g., and V is the volume of the solution. Extinction coefficients for this calculation fall into two groups, depending upon the time and temperature of drying. If the blood meal has been heated for over 7 hr. in steam-jacketed dryers at over 55 lb. pressure the extinction coefficient 3·6 should be used; if the product has been heated for less than 7 hr. at less than 55-lb. pressure or if the treatment is unknown, the coefficient 5·4 should be used. The result thus obtained represents the minimum amount of haemoglobin in the sample; the correct value may be 50 per cent. higher. When blood meal of known haemoglobin content was added to meat and bone scraps also of known haemoglobin content the recovery of haemoglobin ranged from 93 to 104 per cent., with an average recovery of 98·3 ± 2·3 per cent.

The amount of blood in a sample may be estimated as blood meal instead of as haemoglobin from the average haemoglobin content of blood meal, *viz.*, 76 per cent., but this procedure can provide only an approximate figure. The amount of haemoglobin in meat scrap "as might occur unavoidably in good factory practice" remains to be determined. More data are necessary, but the results of this investigation suggest that 1·0 or 1·5 per cent. may be the limit. Values of 2 per cent. or more must be due to careless separation of the blood or to actual adulteration to raise the protein content. The two values for the extinction coefficient of the haemoglobin in blood meal may be used as measures of the heat treatment that any given sample of blood meal has received, and, if it is true that blood meal prepared at high temperatures has a decreased biological value, the extinction coefficient is a measure of the biological value of a given sample. A. O. J.

Fatty Acids of Corn (Maize) Oil. F. J. Baur, jun. and J. B. Brown (*J. Amer. Chem. Soc.*, 1945, **67**, 1899–1900)—Corn oil fatty acids were methylated, without previous separation into saturated and unsaturated fatty acids, and the esters were fractionated. The composition of the several fractions was calculated from iodine values, molecular weights, and thiocyanometric equations. From these results the percentage composition of the acids was calculated as: myristic, 0·1; palmitic, 8·1; stearic, 2·5; hexadecenoic, 1·2; oleic, 30·1; linoleic, 56·3; above C_{18}, 1·7. The linoleic acid content is considerably higher than that obtained by Baughman and Jamieson (*Ibid.*, 1921, **43**, 2696, ANALYST, 1922, **47**, 171), whose calculations of percentage of fatty acids gave: palmitic, 7·8; stearic, 3·6; arachidic, 0·4; lignoceric, 0·2; oleic, 46·3; linoleic, 41·7. These workers separated the saturated and unsaturated acids of corn oil by the lead salt - ether method and based their calculations on iodine and saponification values and on the bromine derivatives of the unsaturated acids. Similarly, Longenecker (*J. Biol. Chem.*, 1939, 9, 13), who separated the saturated and unsaturated acids by a modified Twitchell method and fractionated the methyl esters from each group, obtained only 34·0 per cent. of linoleic acid; the other fatty acid percentages obtained were: myristic, 1·7; palmitic, 11·0; stearic, 2·9; hexadecenoic, 1·6; and oleic, 48·8. E. B. D.

Microscopical Differentiation of Some Common Mustard Seeds with Polarised Light. G. L. Keenan (*J. Assoc. Off. Agric. Chem.*, 1946, **29**, 173–175)—The common commercial mustard seeds resemble each other structurally in transverse section, especially in the epidermal layer of mucilage cells. This mucilage is particularly noticeable when surface sections are mounted in 50 per cent. glycerol, and examined between crossed Nicols. In some species the mucilage is arranged round a central core in the cell, as in white mustard (*Brassica alba*), in Montana brown and Montana oriental mustard (*B. juncea*), and in charlock (*B. arvensis*). In sarepta or brown mustard (*B. besseriana*) the core is not so noticeable and it appears to be absent from black mustard (*B. nigra*).

The polarisation crosses are much larger, sharper, more refractive, and more numerous in white mustard than in the other species, and the arms of the cross measure about 48 μ. Montana brown and Montana oriental mustards show less numerous, less sharply delineated, smaller crosses with arms measuring about 30 μ. In sarepta or brown mustard the crosses are infrequent and not as large as those of white mustard. No crosses are shown by the mucilage cells of black mustard. In charlock the crosses are infrequent and the arms measure about 30 μ. In this species the contents of the cells of the palisade layer are coloured red by acidified chloral hydrate solution. A. O. J.

Determination of Sulphathiazole and other Sulpha Drugs in Honey. C. E. Shepard and H. J. Fisher (*J. Assoc. Off. Agric. Chem.*, 1946, **29**, 175–177)—Sulphathiazole has recently been introduced for the treatment of bees in hives affected with foul brood, the drug being administered in a 50 per cent. sugar solution containing 0·5 g. per gallon. Some of the thiazole appears in the honey and as the amount is of the order of a few parts per million, the colorimetric method of Bratton and Marshall (*J. Biol. Chem.*, 1939, **128**, 537; ANALYST, 1939, **64**, 524) as modified by Gordon and Dunn (*Biochem. J.*, 1941, **35**, 1231) for determination of sulpha compounds in urine was adapted to their determination in honey.

Procedure—Mix 10 g. of the honey with 50 ml. of water and 5 ml. of 4 *N* hydrochloric acid and dilute to 100 ml. In 10 matched test tubes place 1, 2, 3, . . . 10 ml., respectively, of the standard sulphathiazole solution (*infra*) and dilute the contents of each tube to 10 ml. with a solution prepared by diluting 18 ml. of 15 per cent. trichloroacetic acid to 100 ml. For a blank determination place 10 ml. of the diluted trichloroacetic acid solution in another tube. These 11 tubes then contain 0, 3, 6, 9, . . . 30 μg. of sulphathiazole, respectively. In another tube place 10 ml. of the honey solution (≡1 g. of honey) and develop the colour in the sample and standard tubes at the same time by the following procedure.

Add 1 ml. of freshly prepared, 0·1 per cent. sodium nitrite solution and, after 3 min., add 1 ml. of 0·5 per cent. ammonium sulphamate solution and, after a further 3 min., add 1 ml. of 0·1 per cent. N-(1-naphthyl)-ethylenediamine solution. By comparing the sample tube with the standard tubes estimate the amount of sulphathiazole present to the nearest μg.

The red colour develops immediately, is relatively intense, and is stable for several hours. The method could probably be developed as a spectrophotometric method, but this might require filtration of the honey solution. With the honeys used, visual comparison with the series of standard solutions was simpler, filtration was unnecessary, and no interference from the colour of the honey itself was noted at the dilution used. Should other sulpha drugs be used it would be necessary only to substitute these for sulphathiazole in the standard solutions. In honeys from eight hives in which sulphathiazole had been fed to the bees the amount in the honey ranged from 1 to 20 p.p.m. In samples from six hives not subjected to this treatment no sulphathiazole was found in five, and the trace of red colour given by the sixth corresponded to a sulphathiazole content of less than 0·1 p.p.m.

The stock standard solution contains 200 mg. of sulphathiazole in 1 litre of water. To prepare the working standard solution dilute 5 ml. of the stock solution to 100 ml., mix 30 ml. of this solution with 18 ml. of 15 per cent. trichloroacetic acid solution, and dilute to 100 ml. Each millilitre of this solution contains 3 μg. of sulphathiazole. A. O. J.

Microchemical Detection of Vanillin and Bourbonal. A. M. G. Rutten (*Chem. Weekblad*, 1946, **42**, 95, 119)—Most of the microchemical reactions for vanillin and bourbonal are unsatisfactory, especially in presence of other aromatic aldehydes. The following method is claimed to give a sensitive and trustworthy test for the two compounds.

Procedure—Add one drop of the solution to be tested to a drop of 0·05 per cent. aqueous solution of sulphanilic acid on a microscope slide, and allow the liquid to dry in air. Bourbonal gives yellow starry rosettes and "wheatsheaf" bundles; vanillin forms thin branching crystals. Esters, aldehydes, phenols, and colouring matter do not interfere. For essences containing glycerol, or vanilla sugar, extract the material with a small amount of light petroleum, add a drop of sulphanilic acid solution and a few drops of 96 per cent. alcohol, and allow to evaporate on a watch glass. The reaction occurs between two molecules of sulphanilic acid and one of the aldehydic compound. The coloration disappears, reversibly, above 80° C. Photographs of the crystalline forms are given. G. M.

Effect of the Anaesthetic and the Rate of Injection of Digitalis upon its Lethal Dose in Cats. H. G. O. Holck, K. K. Kimura, and B. Bartels (*J. Amer. Pharm. Assoc., Sci. Ed.*, 1946, **35**, 366–370)—It has been reported that different anaesthetics employed in the biological assay of digitalis by the cat method influence the minimum lethal dose of this substance. The fatal dose is reported to be 19 to 20 per cent. higher after urethane than after ether (Edmunds, Moyer, and Shaw, *Ibid.*, 1937, **26**, 290; Holck, Smith, and Shuler, *Ibid.*, 1945, **34**, 90), but conflicting statements exist that the two anaesthetics do not differ in effect in this application (David and Rajamanickam, *Quart. J. Pharm.*, 1934, **7**, 36).

Cats were used anaesthetised with urethane or ether, and were injected with different concentrations of digitalis in parallel experiments.

The dilutions were 1·60, 2·26, 3·20, 4·53, 6·40, and 9·05 per cent. of U.S.P. Digitalis Reference Standard (1942) Tincture, 24 cats being used at each concentration. The results were expressed as the lethal doses per kilogram of cat. The hearts were weighed in addition, but the gain in accuracy did not compensate for the extra work involved.

The two curves relating the logarithms of the median lethal doses (mg. per kg.) to the concentrations of the corresponding digitalis solutions, after either urethane or ether anaesthesia, were parallel.

The less concentrated digitalis preparations gave smaller values for the median lethal dose uniformly throughout the concentration range studied. At equal concentrations of the injection material the lethal dose of digitalis was 6·5 per cent. greater with urethane than with ether anaesthesia. The difference was statistically significant.

The concentration of digitalis with which the lethal dose in cats was smallest was 1·76 per cent. of the standard tincture. When weaker or stronger concentrations were used, larger doses were needed to cause cardiac arrest.

The data presented are statistically analysed and show the errors to be of the same magnitude using either urethane or ether anaesthesia. R. H. T.

Erratum: July issue of 1947, p. 310: In the heading of the first abstract the name of the third author should be, not "J. Warren," but "J. W. Sackett."

Biochemical

Estimation of Ethyl Alcohol in Blood. J. Rochat (*Helv. Chim. Acta,* 1946, **29**, 819–830)—The original method, introduced by Nicloux, gives results that may be in error by as much as 10 per cent. Subsequently, Nicloux published a modified method (*Bull. Soc. Chim. biol.*, 1931, **13**, 857), designed for use in research work demanding greater accuracy, but the older method has nevertheless continued in use for medico-legal work, mainly because the newer method proved to be more complicated. A modification has now been devised that is simple to operate and gives more accurate results than the original method.

Procedure—Put 5 to 15 g. of the fresh blood into a 300-ml. flask, and add 6·5 volumes of a saturated solution of picric acid. If the blood is clotted, break up the clot with a spatula or scissors and add it to the flask. Close the flask with a rubber bung, shake carefully, and leave overnight. Attach the flask to a distillation apparatus of the type described by Nicloux (*loc. cit.*), with a tin condenser to which is attached a glass tube dipping into a few ml. of water contained in a 50-ml. graduated cylinder. Distil at ordinary pressure until two-fifths of the contents of the flask have collected in the graduated cylinder. Stopper the cylinder, mix the contents, and note the volume, V ml. Pipette 5 ml. of a solution (approximately 0·02 *N*) of Mohr's salt (dissolve 7·8 g. of the solid ferrous ammonium sulphate crystals in 1 litre of water containing 20 ml. of concentrated sulphuric acid) into a conical flask, add 40 to 50 ml. of water, and a few drops of concentrated sulphuric acid, and then add from a micro-burette 0·1 *N* potassium permanganate until a permanent pink tinge is produced. Record the volume *a*. Into another flask, pipette 5 ml. of the solution of Mohr's salt, add 0·50 ml. of potassium dichromate solution (8·475 g. per litre) from a micro-burette, followed by water and sulphuric acid as before, and titrate the excess of Mohr's salt with 0·1 *N* potassium permanganate solution. Record the volume *b*. Each titration should be carried out at least twice, and the average used in the calculations. Then 0·50 ml. of potassium dichromate solution is equivalent to $(a - b)$ ml. of 0·1 *N* potassium permanganate solution.

Carry out a preliminary titration of the distillate. Pipette 5 ml. into a 150-ml. beaker, add 6 to 7 ml. of concentrated sulphuric acid and then run in rapidly, from a micro-burette, potassium dichromate solution until a distinct yellowish colour persists. Note the volume. Repeat the oxidation on another 5-ml. portion of the distillate, adding first a volume of potassium dichromate solution equal to that found in the preliminary experiment, and then a volume of concentrated sulphuric acid equal to that of the combined volumes of the test and dichromate solutions plus 1·5 ml. The acid is added down the side of the beaker and mixed by a rapid rotatory movement. Leave for 25 to 30 sec., dilute with about 50 ml. of water, and pipette 5 ml. of the solution of Mohr's salt to reduce the excess of potassium dichromate, and titrate back with 0·1 *N* potassium permanganate. If *c* and *d* are the volumes of potassium dichromate and potassium permanganate solutions, then the volume (*u*) of potassium dichromate solution used to oxidise the alcohol plus oxidisable impurities is equivalent to

$$c - [(a - d)/2(a - b)]$$

Repeat the distillation using water instead of the sample of blood, and oxidise 5 ml. of the distillate as described above. If *r* is the volume of potassium dichromate solution used, then the volume used to oxidise the alcohol only is $(u - r)$ and the concentration of alcohol in the blood (ml. per kg.) $= \frac{u - r}{2} \times \frac{\text{V}}{\text{wt. of blood}} \times F$, where F is an empirical factor which, for aqueous solutions, is 0·97; for fluorinated blood, 0·98; and for coagulated blood, 0·99. The method gives results with an error of only 1 to 2 per cent. Qualitative tests for acetone, aldehydes, etc. may be carried out on portions of the distillate. If acetone is present, satisfactory determinations of alcohol can be made by a modification of the method described, in which the time allowed for the reaction and the excess of dichromate used are reduced.

The method can also be used, with modifications, for the estimation of alcohol in only 1 g. of blood. Transfer the sample, with the aid of 4 ml. of water and 10 ml. of saturated picric acid solution, to a 50- or 100-ml. distillation flask. Distil 4 to 5 ml. through a micro-condenser of tin, the end of which dips into a small volume of water. Distil another 4 to 5 ml., interrupting the heating 2 or 3 times to allow the distillate to be sucked up into the condenser; on resuming the heating, the liquid is forced back into the receiver, thus rinsing out the condenser. Measure the volume of distillate, and to a 2-ml. portion add the requisite volume of potassium dichromate solution (3·39 g. per litre), followed by a volume of concentrated sulphuric acid equal to the combined volumes of the dichromate and test solutions plus 1 ml. Add a solution of Mohr's salt (0·01 *N*) and titrate the excess with 0·02 *N* potassium permanganate. Carry out a blank determination and calculate the result as described above. F. A. R.

Chromatographic Adsorption of Amino-Acids on Organic Exchange-Resins. C. S. Cleaver, R. A. Hardy, and H. G. Cassidy (*J. Amer. Chem. Soc.*, 1945, **67**, 1343–1352)—Several attempts have been made to separate amino-acids by adsorption on organic exchange resins, generally with the solution of a specific problem in mind. In the present paper, an examination is made of the fundamental problems of the exchange process with the object of clarifying the mode of operation of the organic exchange resins in their application to the amino-acids. Two resins were used, a cation exchanger, Amberlite IR–100, and an acid-binding resin, Amberlite IR–4. The former was conditioned by being washed successively with water, 5 per cent. hydrochloric acid solution until the effluent was acid, water, 5 per cent. sodium carbonate solution or aqueous ammonia until the effluent was basic, and then water; this cycle was repeated two to four times until no coloured material was given off at any time during the last cycle. Amberlite IR–4 was conditioned in a similar way with water, 5 per cent. hydrochloric acid solution, water, 5 per cent. sodium carbonate solution or 2 per cent. sodium hydroxide solution, and then water; five or six cycles were usually necessary. With IR–100 the cycle was stopped at the point that gave the desired resin: (H^+R^-), ($NH_4^+R^-$) or (Na^+R^-). The Amberlite IR–4 was left at the point where it was activated by sodium carbonate or hydroxide, symbolised thus: IR–4 (*Na_2CO_3), and IR–4 (*NaOH).

The hydrogen resin, IR–100 (H^+R^-), produced an equilibrium *p*H of 1·9 to 2·9 whatever the *p*H of the amino-acid solution, whilst the sodium resin, IR–100 (Na^+R^-), had less effect on *p*H. At lower equilibrium concentrations the hydrogen resin adsorbed more, and at high equilibrium concentrations less, amino-acid than did the sodium resin. The smaller is the amount of resin used, the greater is the specific adsorption, a result that is inconsistent with simple adsorption and is presumably associated with the exchange. The resin (H^+R^-) appeared to behave like a strong, but very insoluble, acid.

The behaviour of the resins when amino-acid solutions percolated downwards through columns of the resins in a glass tube was studied. Two or more amino-acids were used, one not adsorbed and the other adsorbed. The one that was not adsorbed was used to estimate the amount of liquid initially in the column, whilst the other indicated the exchange capacity of the resin. The two together showed whether the resin would be suitable for separating the mixture. The most convenient particle size was from 60- to 80-mesh. No advantage resulted from the use of a long, narrow column, as compared with a shorter, wider column containing the same weight of resin. The higher is the flow rate, the earlier is the break-through of amino-acid, but the concentration of the amino-acid solution could be varied over a ten-fold range without affecting its behaviour on the column.

The adsorption of histidine in presence of other amino-acids was studied in detail. When alanine was present, histidine was not quantitatively retained on the ammonium resin, even in the first 10 ml. of percolate. The reason for this is that the ammonium resin tends to yield a nearly neutral solution when it acts as an exchanger, and under this relatively low hydrogen ion concentration the histidine is not maintained wholly in the cationic form and therefore not in a state to be completely adsorbed by the ammonium ion. In presence of glutamic acid, however, histidine was retained more strongly by the resin, since the *p*H of the solution is lower than with alanine. A similar difference in behaviour was observed with the sodium resin. When ternary mixtures were investigated, it was found that both histidine and arginine were well adsorbed on ammonium resin in presence of glutamic acid; the histidine leaked through slightly, but both amino-acids broke through at the same point.

Following these preliminary experiments, an attempt was made to separate a mixture of a basic, an acidic, and a neutral amino-acid. A solution (200 ml.) of arginine hydrochloride (1·0 g.), glutamic acid (0·6 g.), and glycine (0·34 g.) was passed through 10 g. of IR–100 (Na^+R^-) at the rate of 2 ml. per min. The column was washed with three, 10-ml. portions of water and the entire percolate was collected. The *p*H of this solution was adjusted to 3, and the solution made up to 250 ml. Of this solution, 200 ml. were passed through 6 g. of IR–4 (*NaOH) at the rate of 2 ml. per min. and the column was washed with three, 10-ml. portions of water and the percolate made up to 200 ml. The IR–100 retained virtually all the arginine and held back practically none of the other two amino-acids. The second column retained 60 per cent. of the glutamic acid and no glycine. An attempt to separate glutamic and aspartic acids on IR–4 was not successful. F. A. R.

Separation and Determination of Primary, Secondary, and Tertiary Alkaryl Amines (Ring-methylated and Nitrogen-methylated Homologues of Aniline). W. Seaman, A. R. Norton, J. T. Woods, and H. N. Bank (*J. Amer. Chem. Soc.*, 1945, **67**, 1571–1578)—The method of Hinsberg (*Ber.*, 1890, **23**, 2962) was found to be unsatisfactory for the separation of aniline and its homologues, and an improved method was developed in which the toluenesulphonamides of the primary and secondary amines are formed and separated from unreacted tertiary amines. The tertiary amines are estimated by extraction from a chlorobenzene solution by means of hydrochloric acid, whilst the sulphonamides of the primary and secondary amines are hydrolysed separately, and the liberated amines are titrated potentiometrically in acid solution by means of standard alkali.

Procedure—Reaction with p-*toluene sulphonyl chloride*—To 6 to 7 g. of the sample in a dry, 250-ml.

flask, add 50 ml. of benzene. Cool in ice, and rapidly add 20·0 g. of *p*-toluene sulphonyl chloride in 25 ml. of dry pyridine. Leave for 30 min. in a stoppered flask in the ice-bath, and then remove the stopper, rinse it with benzene, and attach a reflux condenser to the flask. Pour 10 ml. of water in small portions at a time down the condenser, while cooling the flask in ice. Leave for 30 min. in the ice-bath.

Separation of tertiary from primary and secondary amines—Transfer the reaction mixture quantitatively by means of water and benzene to a 2-litre flask and add 100 ml. of 5 *N* sodium hydroxide. Steam-distil, collecting the distillate under 30 ml. of concentrated hydrochloric acid until 500 ml. have collected. Test the last runnings for amine, to ensure that distillation is complete, by making alkaline and observing whether the solution becomes turbid. Transfer the distillate to a separating funnel, add concentrated hydrochloric acid until acid to Congo red paper and remove the lower aqueous layer. Wash the benzene layer with two, 50-ml. portions of *N* hydrochloric acid and add the washings to the aqueous layer (Solution A). Save the benzene layer for subsequent use (Solution B).

Separation of sulphonamides—Leave the alkaline residue from the steam-distillation in the flask until the oil has settled, and decant the aqueous liquid through a filter paper; if the filtrate is turbid, repeat the filtration. Heat the oil nearly to boiling with *N* sodium hydroxide with shaking, and cool in ice. Filter through the same filter paper as before and repeat the procedure with 200 ml. of *N* sodium hydroxide and several 100-ml. portions of water until a 25-ml. portion of the filtrate does not become turbid or opalescent on acidification. Wash any oil from the filter paper into the flask with hot water and save the filter paper.

Estimation of sulphonamides of primary amines—Transfer the combined alkaline filtrates and washings to a separating funnel and acidify to Congo red paper with concentrated hydrochloric acid. Extract the liberated sulphonamides once with 100 ml., and twice with 50 ml. of benzene. Dry the extracts over anhydrous calcium sulphate and transfer quantitatively through a filter into a tared, 250-ml., glass-stoppered flask containing boiling-chips. Distil off the benzene and dry the residue at 110° C. to constant weight. This gives the weight of sulphonamides of primary amines (W_p).

Estimation of sulphonamides of secondary amines—Transfer the oil by means of Solution B to a 500-ml. separating funnel. Rinse the flask several times with benzene and water, and macerate the filter paper with two or three 50-ml. portions of benzene. Add the rinsings to the separating-funnel, shake, and remove the aqueous layer. Wash the latter with 35 ml. of benzene and add the washing to the main benzene layer. Dry over anhydrous calcium sulphate, filter into a tared, 250-ml. flask, and evaporate and weigh as described above. This gives the weight of sulphonamides of secondary amines (W_s).

Regeneration of primary and secondary amines—To the flasks containing the sulphonamides, add 5 ml. of diluted hydrochloric acid (1 + 1) per g. of sulphonamide, and heat under reflux until the oily layers dissolve or become crystalline (75 to 100 hr.). Test for completeness of hydrolysis by diluting a 1-ml. portion with 10 ml. of water; a turbidity indicates that hydrolysis is incomplete. Dilute the reaction mixture with 10 to 15 volumes of water, transfer with the aid of water and benzene to a separating funnel and, if necessary, extract with two, 100-ml. portions of benzene to dissolve unsaponified sulphonamide. Extract the combined benzene extracts with two, 35-ml. portions of *N* hydrochloric acid and add the acid extracts to the main solution. Dry the benzene solution over anhydrous calcium sulphate, dry, and weigh as before. This gives the weights of unhydrolysed sulphonamides of primary amines (W_{pu}) and secondary amines (W_{su}). Treat the combined acid solution with 5 *N* sodium hydroxide until only slightly acid, cool in an ice-bath, transfer to a 500-ml. separating-funnel, and make strongly alkaline with 5 *N* sodium hydroxide. Extract the amines with three, 100-ml. portions of benzene, excluding any insoluble material. Extract the combined benzene extracts with three 100-ml. portions of *N* hydrochloric acid, again excluding insoluble matter as far as possible. Transfer the acid extracts to a beaker and add 5 *N* sodium hydroxide until the *p*H is 1·0. Boil gently for 5 min. to expel carbon dioxide, cool to room temperature, and saturate with a small excess of sodium chloride. Titrate the solution with *N* sodium hydroxide to *p*H 1·8 and then take *p*H readings after the successive addition of 0·2-ml. portions of alkali until a *p*H of 3·5 is reached. Now add *N* sodium hydroxide to *p*H 6·0 and again take readings after addition of 0·2 ml. portions of alkali to *p*H 10. One inflection (*J*) occurs at about *p*H 2·5 and a second (*K*) at about *p*H 7. Record the mg.-equivalents of alkali (J_p and K_p) consumed at the first and second inflection points, respectively, for the primary amines and the corresponding values (J_s and K_s) for the secondary amines. Then the number of mg.-equivalents of regenerated primary and secondary amines equals, respectively, $L_p = K_p - J_p$ and $L_s = K_s - J_s$. From these values and the weight of sulphonamides, the percentage weight of amines can be calculated. A correction must, however, be applied for the unhydrolysed sulphonamide and, for this, the nature of the unhydrolysed sulphonamide must be known. In the present work, the unhydrolysed sulphonamide of the primary amine was mainly *p*-toluene sulphonmesidide and that of the secondary amine *p*-toluene sulphonmonomethyl-*p*-toluidide, with molecular weights of 289·4 and 275·4, respectively. The corrections in terms of mg.-equivalents are therefore $K_p = 1000W_{pu}/289{\cdot}4$ and $K_s = 1000W_{su}/275{\cdot}4$, respectively, and

Percentage of primary amines =

$$\frac{100W_p - 15{\cdot}42\ [L_p + (1000W_{pu}/289{\cdot}4)]}{\text{Wt. of sample in grams}}$$

Percentage of secondary amines =

$$\frac{100W_s - 15{\cdot}42\ [L_s + (1000W_{su}/275{\cdot}4)]}{\text{Wt. of sample in grams}}$$

Estimation of tertiary amines—Transfer Solution A, after cooling, to a separating-funnel, make alkaline with 5 N sodium hydroxide and, extract once with 150 ml., once with 75 ml., and three times with 50-ml. portions of ether. Dry the combined ethereal extracts over anhydrous calcium sulphate, filter, and transfer to a tared flask containing boiling-chips. Distil off the ether, add 75 ml. of pure chlorobenzene, and distil the mixture carefully through an efficient fractionating column until the b.p. of chlorobenzene (132° C.) has been reached and an additional 10 to 15 ml. of distillate have collected. Weigh the flask to obtain the weight (W_C) of the chlorobenzene solution of the tertiary amine (Solution C). Prepare an apparatus consisting of a glass bulb of convenient size joined by means of a glass tube with a graduated scale to a second larger bulb, through the top of which liquid can be introduced by means of a small funnel. Calibrate the scale in terms of weight of chlorobenzene by putting into the apparatus known weights of chlorobenzene, adding 1 ml. of N hydrochloric acid, extracting as in the estimation of tertiary amines and noting the scale reading. Fit the dry, calibrated apparatus with a cork stopper, and weigh. Put sufficient of Solution C into the apparatus to come to the top of the scale and weigh the apparatus again to obtain the weight of Solution C taken (W_Q). Now add 15 ml. of N hydrochloric acid, shake for about 5 min., and centrifuge. Immerse in a thermostat at 25·0 ± 0·2° C. for 30 min., and record the scale reading corresponding to the interface. Repeat the extraction with fresh 15-ml. portions of N hydrochloric acid until constant differences are observed in successive scale readings; 3 or 4 extractions are generally necessary. This constant difference is caused by the slight solubility of chlorobenzene in N hydrochloric acid. Let f be the correction factor for this solubility, n be the number of 15-ml. portions of hydrochloric acid used, and h be the first scale reading at which constant differences are obtained, then h (corr.) $= h + (f \times n)$. From the calibration constants of the apparatus, calculate the weight of chlorobenzene (W_E) corresponding to the scale reading h (corr.). Then $W_Q - W_E$ is the weight of tertiary amine in the weight W_Q of the chlorobenzene solution, and percentage of tertiary amines =

$$\frac{W_C\ (W_Q - W_E)}{W_Q \times \text{wt. of sample in grams}} \times 100$$

Where the method was used for the estimation of known amounts of primary, secondary, and tertiary amines in six different mixtures, the error exceeded ± 2·3 per cent. in only 2 analyses out of 18.

F. A. R.

Organic

Quantitative Method for Aconitic Acid and Aconitates. E. J. Roberts and J. A. Ambler (*Anal. Chem.*, 1947, **19**, 118–119)—Balch, Broeg, and Ambler (*Sugar*, 1945, **40**, 32; 1946, **41**, 46) found that dilute, aqueous solutions of aconitic acid evolve carbon dioxide during evaporation by heat at normal temperatures and pressures, but the decarboxylation proceeds too slowly for analytical purposes. It was observed that, although aconitic acid is stable in hot, glacial acetic acid, some aconitates evolve carbon dioxide when boiled in this medium, the decarboxylation being very rapid with the soluble alkali aconitates, slow with the slightly soluble cadmium, calcium, and calcium-magnesium salts and extremely slow with the very insoluble lead salt. When, however, an excess of potassium acetate is added to suspensions of the more insoluble aconitates in acetic acid, and the mixtures are heated to boiling point, decarboxylation proceeds rapidly and is complete in from 30 to 40 min., each molecule of aconitic acid (or each aconitate radicle in an aconitate) yielding 1 molecule of carbon dioxide according to the reaction $C_6H_6O_6 \rightarrow CO_2 + C_5H_6O_4$. This principle can be applied to the determination of aconitic acid and aconitates.

The apparatus consists of a 250-ml. Erlenmeyer flask heated by an electric plate and connected with a tube through which a stream of carbon-dioxide-free air is introduced into the flask above the level of the liquid and with a short reflux condenser, the top of which is connected with a wash-bottle containing water for removing acetic vapour from the gas and air mixture. The wash bottle, in turn, is connected with a suitable carbon dioxide absorption train.

Procedure for dry solids.—To 1 to 2 g. of aconitic acid or 2 to 5 g. of aconitate in the flask add 75 to 100 ml. of "empyreuma free" glacial acetic acid, some carborundum chips, and 5 or 10 g. of potassium acetate, using the larger amount for insoluble aconitates. Connect the flask with the air inlet tube and with the condenser, attach the wash-bottle, and pass a slow stream of carbon-dioxide-free air through the system for 15 to 20 min. Start heating the flask and attach the carbon dioxide absorption train and maintain the passage of the slow stream of air while the reaction mixture is heated under reflux for 1 hr. Then disconnect the absorption train and determine the carbon dioxide absorbed.

Procedure for aqueous solutions—Dilute an amount of solution containing from 0·5 to 2·0 g. of organic acid, calculated as aconitic acid, to about 200 ml. with carbon-dioxide-free water, adjust the *p*H of the solution to 6·0 to 6·2 with either acetic acid or sodium hydroxide solution and add, with stirring, 50 ml. of saturated, neutral lead acetate solution. Collect the precipitate on a strong filter paper fluted to fit a Hirsch or a small Buchner funnel and pre-coated with a thin layer of finely divided asbestos. Wash the paper and precipitate once with water and drain completely by suction. Discontinue the suction and fill the paper with acetone, carefully manipulating the precipitate so that the acetone penetrates it thoroughly. Drain off the acetone and repeat the extraction. Finally, drain the acetone through by suction and maintain the suction until the paper is dry. Dry the precipitate and paper at 100° to 105° C. for 0·5 hr. Place the paper and precipitate in the decarboxylation flask, add 100 ml. of acetic acid, 10 g. of potassium acetate, and some carborundum chips and proceed as directed for dry solids.

The carbon dioxide may be absorbed and determined by any standard method. In the work reported it was determined volumetrically by precipitation of the carbonate with barium chloride solution and titration of the residual alkalinity according to Reid and Weihe's modification (*Ind. Eng. Chem., Anal. Ed.*, 1938, **10**, 271) of Winkler's method. For this and similar volumetric methods 1 ml. of *N* sodium hydroxide ≡ 0·087 g. of aconitic acid; for gravimetric methods, 1 mg. of carbon dioxide ≡ 3·954 mg. of aconitic acid.

If carbonates or bicarbonates are present, acidify the solution strongly with acetic acid and remove carbon dioxide by aeration at room temperature. Neutralise the solution and proceed with the precipitation and decarboxylation of the lead salt as already described. When solid aconitates are contaminated with carbonates, two determinations of carbon dioxide are necessary. For total carbon dioxide, set up the apparatus with a separating funnel delivering into the decarboxylation flask. Proceed as already described, but at the end of the decarboxylation period allow the reaction mixture to cool until refluxing stops, keeping the absorption train attached and maintaining the current of air at such a rate as to prevent backward suction. Add 100 ml. of carbon-dioxide-free water through the funnel and heat the diluted mixture under refluxing conditions for a further 30 min. to ensure complete decomposition of the carbonates. Determine the total carbon dioxide evolved and calculate it as per cent. of aconitic acid. Determine the carbon dioxide from the carbonates by dissolving a second portion in hydrochloric acid and aerating at room temperature for 1 hr. (MacIntire and Willis, *J. Ind. Eng. Chem.*, 1915, **7**, 227; ANALYST, 1915, **40**, 251), calculating the carbon dioxide obtained as per cent. of aconitic acid. For the actual aconitic acid in the sample deduct this value from the value obtained from the modified decarboxylation procedure.

Water retards the reaction and with alcohol low results are obtained, probably owing to esterification of some of the aconitic acid. Malonic acid and acetonedicarboxylic acid are rapidly decarboxylated, the latter acid yielding two molecular proportions of carbon dioxide. The presence of acids of these two types has not been reported in sorgo or sugar cane. Galacturonic acid yields 53 per cent. of its carbon dioxide in 1 hr. Since uronic acids are normal constituents of plants and have been reported in sugar cane, the extent of this interference will receive further investigation. Oxalic, succinic, maleic, fumaric, itaconic, citraconic, mesaconic, malic, lactic, tartaric, tricarballylic, glutamic, aspartic, aminoacetic, and phthalic acids do not interfere. Citric acid and some citrates yield small amounts of carbon dioxide under certain conditions, and the extent of their interference will be investigated. Cystine gives no carbon dioxide, but the solution darkens and evolves hydrogen sulphide. Carbohydrates do not yield carbon dioxide in the procedure. Salts of easily volatile inorganic acids (chlorides, nitrates, and carbonates) yield acid vapours which pass into the absorption train. Hydrogen chloride from chlorides can be trapped effectively by washing the gas with silver nitrate or sulphate solutions. Nitrates, even in small amounts, interfere both by evolving acidic oxides of nitrogen and by oxidising the aconitic acid and the decarboxylated residue with evolution of some carbon dioxide. Hence the method is not applicable in presence of nitrates. Copper aconitate when tested by the method gives excessive and variable results, and cuprous oxide appears in the reaction mixture. For this reason the method is not applicable to material containing cupric, silver, mercuric, or other oxidising compounds that are soluble in the hot acetic acid and acetate mixture.

The method gives results of satisfactory accuracy in the assay of laboratory preparations of aconitic acid and aconitates. A. O. J.

Micro-determination of Tetra-ethyl Lead in Gasoline. B. E. Gordon and R. A. Burdett (*Anal. Chem.*, 1947, **19**, 137–140)—The method is based upon the decomposition of the tetra-ethyl lead with iodine, removal of the volatile constituents by evaporation, destruction of the organic residue with mixed sulphuric, nitric, and perchloric acids, and subsequent volumetric micro-determination of precipitated lead chromate. The entire analysis is conducted without transfer of the sample from one flask and thus serious errors are eliminated.

Into a clean, 125-ml. Erlenmeyer flask containing 1 ml. of a saturated solution of iodine in carbon tetrachloride pipette 1 ml. of the sample of gasoline and, at the same time, observe its temperature. If the tetra-ethyl lead content of the gasoline is 1 ml. or less per gallon, use 5 ml. of the sample. Swirl the mixture and evaporate the volatile constituents by gentle heating over a hot-plate, assisting the removal of iodine by tilting the flask and pouring out the heavy vapour. Add 9 ml. of a digestion mixture prepared by mixing 300 ml. of concentrated sulphuric acid, 500 ml. of concentrated nitric acid, and 100 ml. of 72 per cent. perchloric acid, insert a glass bead, and fit the anti-spatter device (*infra*) into the neck of the flask. Boil the contents of the flask on a hot-plate with occasional swirling until the sulphuric acid condenses near the neck of the flask. Remove the flask from the plate, cool slightly, and add 0·5 ml. of perchloric acid through the anti-spatter device. Repeat the boiling until the sulphuric acid condenses near the neck of the flask and continue until the solution is colourless. Allow the flask to cool and, after a short time, accelerate the cooling by immersing the flask in a shallow water-bath. Cautiously add 10 ml. of water through the anti-spatter device and, if the liquid now shows a yellow colour due to residual iodine or chlorine, the boiling must be repeated.

Remove the anti-spatter device, rinsing it with 15 ml. of water, and slowly add down the side of the flask 7 ml. of concentrated aqueous ammonia and 2 drops of 0·5 per cent., aqueous *p*-nitrophenol solution. Cautiously add concentrated aqueous ammonia until 1 drop changes the colour of the liquid to deep yellow, guarding against the appearance of a false end-point. Add diluted acetic acid (1 + 3) until 1 drop removes the strong yellow colour, and then add an excess of 4 drops. Add 3 ml. of 10 per cent. ammonium acetate solution, boil gently on a hot-plate, and, if lead sulphate is evident as a white precipitate, continue boiling until this has dissolved. Add 2 drops of the diluted acetic acid followed by 5 ml. of 5 per cent. potassium dichromate solution, boil for 10 min., and allow to cool for 15 min.

Remove the supernatant liquid by reverse filtration through a Munroe platinum filter-stick connected with a filter flask and suction pump. Wash the precipitate and flask with 5-ml. portions of 1 per cent. ammonium acetate solution, removing the liquid through the filter-stick after each addition. Disconnect the filter-stick and attach it by means of a short length of small-bore pressure tubing to a micro-funnel that has been inserted through one hole of a two-holed stopper. Insert this stopper with the filter-stick attached into the neck of the flask and apply suction through the second hole in the stopper. Draw 2 ml. of water through the funnel and filter-stick into the flask and then, drop by drop, 4 ml. of diluted hydrochloric acid (1 + 3) followed by five, 1-ml. portions of water, and finally remove the stopper without interrupting the suction and rinse the outside of the filter-stick into the flask with water. To the contents of the flask add 1 ml. of the diluted hydrochloric acid and enough water to make the volume 30 ml. To the solution of lead chromate in hydrochloric acid add 0·5 g. of potassium iodide and swirl gently until solution is complete. Titrate the liberated iodine to a light yellow colour with 0·0018 *N* sodium thiosulphate, add 1 ml. of 1 per cent. starch solution, and complete the titration. Effective swirling of the liquid is necessary as the end-point is approached. The tetra-ethyl lead content in ml. per gallon (uncorrected) is given by $(M \times N \times 0{\cdot}1077 \times 2296)/S$, where M is the number of millilitres of sodium thiosulphate solution of normality N used in the titration, 0·1077 is the mg.-equivalent weight of lead chromate, 2296 is the factor for converting lead chromate to ml. of tetra-ethyl lead per gallon, and S is the number of millilitres of sample used. If U is the uncorrected tetra-ethyl lead content found by this calculation, the corresponding figure (ml. per gallon) corrected to 15·5° C. is $0{\cdot}001U(T - 15{\cdot}5) + U$, where T is the observed temperature of the sample taken and 0·001 is the mean cubical coefficient of expansion of gasoline.

It was found that the use of 0·0018 *N* (0·00171 *N* to 0·00191 *N*) sodium thiosulphate rather than 0·002 *N* resulted in greater sensitivity without reduction of precision. Since A.S.T.M. specifications demand a precision of ±0·03 ml. of tetra-ethyl lead per gallon, the method requires rigorous attention to detail throughout: the sodium thiosulphate must be accurately standardised, the semimicro-burettes should be of 10-ml. capacity graduated to 0·05 ml. and readable to 0·01 ml.

The anti-spatter device consists of a wide glass tube ground in to fit the neck of the flask. The walls of the tube have been made to collapse inwards at three points to form three internal nipples one above the other and arranged at points 120° apart on the circumference of the tube so that passage of droplets through the tube is impeded by one or other of the nipples. A. O. J.

Inorganic

Colorimetric Determination of Molybdenum as the Thiocyanate Complex. N. A. Tananaev and A. P. Lochvitzkaja (*Zavod. Lab.*, **1945, 11,** 6–10) —The red coloration obtained with thiocyanates and molybdenum is due to quinquevalent molybdenum, probably in the form $H_2MoO(SCN)_5$, and not to molybdenum in still lower valency states as is usually supposed. This is shown by reducing Mo^{VI} (ammonium molybdate) in diluted sulphuric acid (1 + 3) with zinc amalgam to Mo^{III}, estimating in one aliquot the volume of standard permanganate solution necessary for complete oxidation, and then testing (i) an unoxidised aliquot, (ii) an aliquot oxidised with one-third of the permanganate required for full oxidation, and (iii) an aliquot oxidised with two-thirds of the amount. With potassium thiocyanate in diluted sulphuric acid (1 + 5), aliquot (iii) gives the familiar orange or red colour, aliquot (ii) does not give any colour at first, but air oxidation causes the red colour to appear in time, and after several days Mo^{IV} is completely oxidised to Mo^{V}, and aliquot (i), originally bluish-green, does not assume an orange or red tint until after a much longer time. Bismuth amalgam reduces Mo^{VI} quantitatively to Mo^{V}, which with thiocyanate gives a stable compound not oxidisable by air.

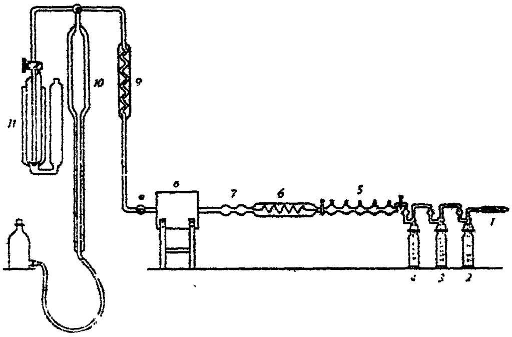
Thiocyanate alone (*i.e.*, without stannous chloride or other reducing agent) reduces Mo^{VI} to Mo^{V}, slowly in the cold, but fairly rapidly in hot solutions, and the reaction may be used for the detection of molybdenum in absence of iron, thus: (i) to the diluted hydrochloric or sulphuric acid solution (1 + 5 to 1 + 7 in either case), heated to boiling, add, after removal from the source of heat, 1 to 3 drops of saturated potassium thiocyanate solution; a coloration, developing after 20 to 30 sec., reaches a maximum after 2 to 3 min., (ii) place a drop of the solution to be tested on a filter paper, add a drop of diluted hydrochloric acid (1 + 1), and then a drop of saturated potassium thiocyanate solution. The development of a yellow, orange, or red colour is proof of the presence of molybdenum if iron is absent. In neutral or weakly acid solution, molybdenum gives no colour with thiocyanate, hence the presence of iron may be detected under these conditions by the formation of the normal ferric thiocyanate colour.

The colour forms in nitric acid solutions, free from nitrous acid, as well as in hydrochloric and sulphuric acid solutions. The test must be carried out in the cold, but stannous chloride should then be added. It is found that the colour is stable at least for one hour; other workers have made contrary statements.

The sensitivity of the reaction when stannous chloride is used is 2 μg. of molybdenum per ml. under the following conditions: to 3 ml. of diluted (1 + 5 to 1 + 7) hydrochloric or sulphuric acid add 1 drop (about 0·05 ml.) of 0·001 *M* ammonium molybdate, 3 drops of saturated potassium thiocyanate solution, and 1 drop of stannous chloride solution (20 g. of $SnCl_2.2H_2O$ and 1 g. of tin heated to solution of the crystals in 15 ml. of concentrated hydrochloric acid, and the solution diluted to 100 ml.).

Determination of molybdenum in steel. Procedure—Dissolve 1 g. in 30 to 40 ml. of diluted sulphuric acid (1 + 5), add concentrated nitric acid until frothing ceases, boil off the reddish-brown fumes, transfer the solution to a 250-ml. graduated flask, cool, add permanganate to give a permanent red colour, dilute to the mark, and mix. Pipette 10 ml. of the solution into a beaker, add 2 ml. of 10 per cent. potassium thiocyanate solution and 4 ml. of 25 per cent. stannous chloride solution, and mix. Compare in a colorimeter with a similarly prepared solution of a standard steel. G. S. S.

New Method for the Determination of Oxygen in Iron and Steel. N. Vigna (*Helv. Chim. Acta.*, 1946, **29**, 1667–1669)—Existing methods for the determination of oxygen in iron and steel, based on heating the sample in hydrogen, are considered to be difficult and unreliable. The proposed method is a modification of the Ströhlein method for carbon. The sample is heated in a current of pure nitrogen and the oxygen is converted to carbon monoxide; the volume of this is determined by absorption in ammoniacal cuprous chloride solution.



Apparatus—Commercial nitrogen is passed over hot copper oxide (1), through alkaline pyrogallol solution (2 and 3), through sulphuric acid (4), and over phosphorus pentoxide (5). To remove any remaining trace of oxygen it is then passed over hot reduced copper (6) and through a glass-wool filter (7) to the combustion tube (8). From the outlet from the combustion tube the gas may be vented to the atmosphere or directed through a cooler (9) to a measuring burette (10), which may be connected to the absorption vessel (11) containing cuprous chloride solution. As the volume of carbon monoxide is small the part of the burette in which the contraction is measured is of 5 mm. bore. The confining liquid in the burette is a saturated solution of potassium chloride, coloured with methylene blue, and saturated with pure nitrogen

Method—The sample is prepared in such a way that oxidation is avoided. This may be achieved by clamping the specimen in a recessed steel block and putting a piece of solid carbon dioxide in the recess during the machining operation. The sample, in a boat, is introduced into the combustion tube and the apparatus is swept with nitrogen. The boat is then heated at 350° C. in a stream of nitrogen to remove occluded air. The sample is finally heated to 1200° C., the gas is collected in the burette and the measurement of the carbon monoxide volume finally made. With some steels it is desirable to mix a small amount of pure carbon with the sample.

The average error of the method is about ±0·01 per cent. L. A. D.

Determination of Hydrogen in Liquid Steel. J. E. Wells and K. C. Barraclough (*J. Iron and Steel Inst.*, 1947, **155**, 27–32)—The paper reviews five methods of sampling a liquid steel bath. The determination is made by heating the sample in a vacuum at 600° C. as described by Newell (*Ibid.*, 1940, No. I, p. 243P). The apparatus for handling small samples has been modified so that the specimens are introduced and removed through a mercury seal without admitting air.

The sampling methods are:—

(*a*) The balloon-tube method of Hatfield and Newell (*Ibid.*, 1943, No. II, p. 407P).
(*b*) The notched-pencil method, also of Hatfield and Newell (*loc. cit.*).
(*c*) The water-chilled mould method.
(*d*) The cast iron chill mould method.
(*e*) The ingot sample.

In the first four methods, a sample spoon is "slagged" and a spoonful of metal withdrawn from the furnace. Any slag is skimmed off, the steel is "killed" with aluminium and the sample prepared. In the fifth method, a 5-cwt. ingot is cast with the other ingots from the heat and sectioned to yield two samples 2¾ in. in diameter and 3 in. long. The machining and analysis must be conducted without delay to avoid loss of hydrogen from the samples.

All the methods give satisfactory results for the hydrogen content, a modification of method (*b*) being considered simplest. The mould is filled and as soon as the metal solidifies it is removed from the mould and quenched in water. If any interval must elapse before analysis the samples are kept in solid carbon dioxide. L. A. D.

Determination of Gallium in Silicate Rocks. E. B. Sandell (*Anal. Chem.*, 1947, **19**, 63–65)—The method for the determination of traces of gallium in silicate minerals and rocks is based on the fluorescence given by gallium with 8-hydroxyquinoline (*Idem.*, *Ind. Eng. Chem.*, *Anal. Ed.*, 1941, **13**, 844). The gallium hydroxyquinolinate is extracted with chloroform from an aqueous solution at *p*H 2·6 to 3·0. The only other metal hydroxyquinolinates that impart a fluorescence to the chloroform in this *p*H range are indium and scandium. At *p*H 3, the fluorescence given by indium is 1/500 that of an equal weight of gallium, and that of scandium 1/40,000 as strong. Coloured hydroxyquinolinates that dissolve in chloroform are formed by ferric iron, quinquevalent vanadium, cupric copper, and sexavalent molybdenum. Iron and vanadium do not interfere when reduced by hydroxylamine hydrochloride. Interference by several of the elements mentioned is avoided by extracting the gallium as its chloride with ether before applying the fluorescence reaction. As the presence of much iron in the ether extract makes the subsequent treatment of the extract more difficult, ferric iron is reduced by finely divided silver before the ether extraction. Traces of copper and vanadium may be extracted by the ether, but the amounts of these metals present in igneous rocks are such as to cause no error. Molybdenum, if present in quantity, would be extracted by the ether and would give a yellow colour to the chloroform.

Reagents—Prepare the silver powder by reducing silver nitrate with metallic copper. Wash well with dilute sulphuric acid and water, and dry. To prepare the silver column place a plug of glass-wool at the bottom of the stem of a funnel, stem diameter 8 mm., and fill the stem loosely to a depth of 15 mm. with powdered silver. After use, rinse the column with diluted hydrochloric acid (1 + 1) and water, and dry.

Use analytical grade ether treated daily as follows. Shake 65 ml. in a separating-funnel with 25 ml. of diluted hydrochloric acid (1 + 1) containing 0·05 g. of sodium hydrogen sulphite. Separate the phases. Do not wash the ether phase, but rinse the stem of the funnel by pouring a small quantity of the diluted hydrochloric acid into the funnel and running it off.

8-Hydroxyquinoline solution. Dissolve 0·1 g. in a small quantity of water containing 0·6 ml. of 6 *N* acetic acid and dilute to 100 ml.

Standard gallium solution. A suitable concentration is 0·0005 per cent. of gallium. The solution should be 0·05 *N* with respect to hydrochloric acid.

Procedure—To 0·25 g. of the 100-mesh rock powder in a platinum dish add 2 ml. of 6 *N* sulphuric acid and 3 ml. of hydrofluoric acid. Evaporate to dryness and fume off the excess of sulphuric acid. Take up the residue in 0·5 ml. of 6 *N* sulphuric acid and 1 to 2 ml. of water, evaporate to dryness, and fume off the sulphuric acid. Warm the residue, stirring at intervals, in a covered dish with 10 ml. of diluted hydrochloric acid (1 + 1) until all soluble material has dissolved, filter off any calcium sulphate, and wash with 5 ml. of water containing a few drops of hydrochloric acid. Collect the filtrate and washings in a 25-ml. graduated flask. The residue from some minerals will require further treatment according to its nature. Add 0·5 g. of silver powder to the contents of the flask and swirl until most of the ferric iron has been reduced; add 8 ml. of concentrated hydrochloric acid and dilute to 25 ml. After mixing for 1 min., run the solution through the silver column and transfer 10 ml. of the effluent to a small separating-funnel previously washed with the diluted hydrochloric acid. Add 8 ml. of ether and shake for 20 to 30 sec. Drain the aqueous phase into another separating-funnel and shake it with 5 ml. of ether. Discard the acid layer and combine the second ether extract with the first, rinsing the funnel with 1 ml. of ether. Shake the combined ether extracts vigorously for 10 sec. with 1 ml. of diluted hydrochloric acid (1 + 1), drain off the aqueous layer and shake again with 1 ml. of the hydrochloric acid. Discard the acid layer. To rinse out the stem, add a few drops of acid to the funnel and draw it off without shaking.

Run the washed ether extract into a 50-ml. beaker containing 0·5 ml. of 10 per cent. sodium chloride solution, and rinse the funnel with 1 to 2 ml. of ether. Evaporate the ether at a low temperature from the covered beaker, remove the watch glass, and allow the water to evaporate. Dissolve the cold residue in 2 ml. of 0·2 *N* hydrochloric acid and transfer the solution to a flat-bottomed, glass-stoppered bottle, 1·8 cm. by 15 cm., of 25-ml. capacity, washing the beaker and cover glass with about 3 ml. of water. Add 1 ml. of 20 per cent. hydroxylamine hydrochloride solution, mix, add 6 ml. of potassium hydrogen phthalate solution (40·82 g. per litre), and allow to stand for 20 min. Prepare a comparison solution of the same composition.

To the sample and comparison solutions add 0·25 ml. of the 8-hydroxyquinoline solution, mix by inversion, and add 2 ml. of analytical grade chloroform. Shake the sample tube vigorously for at least 30 sec. and allow the chloroform to settle. Add portions of the standard gallium solution from a micro-burette to the comparison tube, shaking for 30 sec. after each addition. Compare the fluorescence of the two chloroform layers by holding the two tubes vertically above an ultra-violet lamp in a dark room. When the intensities of fluorescence are nearly the same shake the sample tube for up to 1 min. and then shake both tubes for 30 sec. after each further addition of gallium to the comparison tube. When the intensities are the same, shake both tubes for 1 min. to ensure that distribution equilibrium has been reached. Run a blank determination through the whole procedure.

The procedure was tested on a series of rocks containing from 0·5 to 19 p.p.m. of gallium, further quantities of gallium equivalent to up to 50 p.p.m. being added to some of the samples. The results present indicate that several per cent. of the gallium escape extraction. Application of a correction factor of +10 per cent. is believed to be justified. With this correction, all the results but one are within 10 per cent. of the theoretical results. The blank was equivalent of 0·1 μg. of gallium. B. A.

Agricultural

Determination of the Pyrethrins in Pyrethrum Concentrates in Mineral Oil. J. T. Martin and S. T. P. Brightwell (*J. Soc. Chem. Ind.*, 1946, **65**, 379–384)—For the determination of pyrethrins in extracts of pyrethrum flowers in heavy mineral oil, such as are used for the control of insects that attack stored products, the Wilcoxon-Holaday method (*Assoc. Off. Agric. Chem., Methods of Analysis*, 1940, 67) is modified (*a*) by removing uncombined acids before analysis, by extracting a chloroform solution of the concentrate with dilute baryta, (*b*) by fractionating the saponified concentrate between water and light petroleum to remove oil, (*c*) by extracting the chrysanthemum monocarboxylic acid in the presence of the barium sulphate precipitate, to avoid losses by adsorption of the acid on this precipitate, and (*d*) by twice washing the light petroleum solution with 2·5 ml. of water instead of once with 5 ml. in the recovery of the monocarboxylic acid before treatment with Dénigès' reagent, thus obtaining a clear separation of the aqueous and the light petroleum layers.

DETERMINATION OF PYRETHRIN I. *Reagents. Dénigès' reagent*—Mix 5 g. of yellow mercuric oxide with 40 ml. of water, and, while stirring vigorously, slowly add 20 ml. of concentrated sulphuric acid and then 40 ml. of water. Continue to stir until all the oxide has dissolved, cool, and filter. *Iodine monochloride reagent*—Dissolve 10 g. of potassium iodide and 6·44 g. of potassium iodate in 75 ml. of water in a glass-stoppered bottle. Add 75 ml. of concentrated hydrochloric acid and then 5 ml. of chloroform. Shake well and add solutions of potassium iodide or iodate until the chloroform layer is just pink. Keep in the dark and adjust periodically. *Potassium iodate* (0·01 *M*)—Dry potassium iodate at 105° C. Dissolve 2·14 g. in water and dilute to 1 litre. N *alcoholic* (95 *per cent.*) *sodium hydroxide*—Dissolve 40 g. of caustic soda in 1 litre of 95 per cent. alcohol distilled from *m*-phenylenediamine; or, purify the alcohol by means of silver oxide (Griffin, *Technical Methods of Analysis*).

Preparation of sample—Mix the sample well by shaking, allow to settle until clear, and use the clear solution for analysis. If necessary, remove suspended particles by centrifuging. Take for analysis a sample containing sufficient pyrethrin I to give a titration of the order of 12 to 15 ml. of potassium iodate solution. When this is greatly exceeded, make up to a definite volume the filtrate from the barium chloride precipitation and continue the assay with aliquot portions. Approximate volumes required are 20 ml. of a preparation said to contain 0·8 per cent. of total pyrethrins, or 4 ml. of a preparation said to contain 4·0 per cent. of total pyrethrins.

Procedure—Weigh the sample into a 100-ml. beaker, dissolve in 50 ml. of chloroform, and wash into a 500-ml., pear-shaped separating funnel, using 50 ml. of chloroform. Add 75 ml. of water, 1 drop of 1 per cent. phenolphthalein, and 5 ml. of 0·1 *N* baryta. Agitate the funnel for a minute or two, adding more baryta if the aqueous layer does not remain alkaline. Add 10 ml. of saturated salt solution and 10 ml. of 10 per cent. barium chloride solution, rotate, and allow the chloroform layer to separate. Run the chloroform layer into a second separating funnel containing 25 ml. of water and a few ml. of saturated salt solution, and swirl. Run the chloroform layer into a 250-ml. round-bottomed flask. Wash the aqueous layers successively with four 20-ml. portions of chloroform, shaking vigorously for the third and fourth washings to break any emulsion in the aqueous layers. Wash for a fifth time with 10 ml. of chloroform if the colour of the chloroform separating or the persistence of an emulsion in the aqueous layer indicates that this is necessary. Add the washings to the main chloroform solution. (At this stage, the estimation may be left overnight.) Retain the aqueous layers for the determination of free chrysanthemum monocarboxylic acid when this is required. Distil the chloroform to low bulk from boiling water and remove as completely as possible under moderate vacuum at 35° to 40° C., rotating the flask and using an anti-splash device. Add 20 ml. of *N* alcoholic (95 per cent.) sodium hydroxide, washing out the anti-splash device, saponify for 2 hours, and cool. Add 80 ml. of water, swirl to dissolve the soaps, add 50 ml. of light petroleum, and wash into a 500-ml. pear-shaped separating funnel, using 50 ml. of water and then a few millilitres of light petroleum. Swirl, but do not shake. Run the aqueous layer into 50 ml. of light petroleum in a second separating funnel and again swirl. Draw off the aqueous layer into a 600-ml. beaker with a mark at 150 ml., and wash the light petroleum layers successively with two portions of 25 ml. each of water; if material insoluble in water and light petroleum causes troublesome emulsification at the junction of the two layers, strain the emulsion through a loose plug of cotton wool to break it up. Add the washings to the main aqueous layer. Boil the aqueous layer down to 150 ml., gently at first to avoid frothing. Cool, add a little Celite filter aid and 10 ml. of 10 per cent. barium chloride solution, stir, and allow to stand for at least 10 min. (At this stage the estimation may be left overnight.)

Filter into a 500-ml. separating funnel through a fluted, 12-cm., No. 1 Whatman paper, testing the filtrate with a little barium chloride solution to ensure that precipitation is complete. Wash the beaker and precipitate thoroughly with successive portions, 50 ml. in all, of water. Add 5 ml. of diluted sulphuric acid (1+4) to the filtrate, mix, and test to see that the solution is acid to litmus. Add a few ml. of saturated salt solution and shake vigorously for at least 1 min. with 50 ml. of light petroleum (b.p. below 40° C.). Allow the layers to separate. Draw off the precipitate and aqueous layer into a beaker and run the light petroleum extract through a loose plug of cotton wool into a separating funnel. Repeat the extraction of the aqueous layer with 50-ml. and 25-ml. portions of light petroleum, adding these through the wool to the first extract. Draw off any water that separates and wash the light petroleum solution with 3 portions of 10 ml. each of water, allowing adequate drainage. Retain the aqueous layer and each of the washings (separately) for the determination of pyrethrin II. Extract the light petroleum solution by vigorous shaking with 5 ml. of 0·1 *N* aqueous caustic soda for at least 1 min. Allow the alkaline layer to separate as completely as possible and run it into a 100-ml., lipped conical flask with a ground-glass stopper. Wash the light petroleum solution with two portions of 2·5 ml. of water and add these to the first extract, allowing each to drain as completely as possible. (At this stage the estimation may be left overnight.) Add 10 ml. of Denigès' reagent to the alkaline extract and set aside for 1 hour at room temperature (approximately 20° C.). Add 20 ml. of acetone and 3 ml. of saturated salt solution, stir, heat just to boiling and, after the precipitate has settled, decant through a 7-cm., No. 1 Whatman paper. Wash the precipitate by decantation with 10 ml. of hot acetone, and two portions of 10 ml. each of hot chloroform. Place the drained paper in the flask used for the precipitation, add 30 ml. of concentrated hydrochloric acid and 20 ml. of water, and cool. Add 6 ml. of chloroform and 1 ml. of iodine monochloride solution and titrate, with vigorous shaking, with 0·01 *M* iodate solution until the chloroform has no pink colour. The pink colour will return slowly. If over-titrated, add a definite volume of 0·02 *M* potassium iodide solution and titrate back. Then 1 ml. of 0·01 *M* iodate ≡ 5·7 mg. of pyrethrin I (taking the Graham and LaForge factor for pyrethrin I, *Soap*, 1943, **19**, No. 11, 111).

DETERMINATION OF PYRETHRIN II. Use the aqueous layers retained above. Allow the precipitate in the main layer to settle, decant the solution into a second beaker, boil down to 50 ml., and cool. Filter the solution on a Gooch crucible containing filter paper and a little asbestos, finally washing the precipitate in. Wash the beaker and filter with the three 10-ml. portions of washings retained above. Make the clear filtrate alkaline with sodium bicarbonate, wash into a separating funnel, and extract twice with 25-ml. portions of chloroform. Wash the chloroform extracts successively with two portions of water and combine all the aqueous layers. Discard the chloroform extracts. Acidify the aqueous layer with hydrochloric acid and saturate with sodium chloride, avoiding loss by frothing. Extract thoroughly in a separating funnel with two 50-ml. and two 25-ml. portions of ether (distilled from caustic soda). Combine the ether extracts in a second separating funnel, allow to drain, and wash with two portions of 10 ml. of water, allowing careful drainage each time. Run the ether solution into a 350-ml. conical flask and remove the ether on the water-bath. Allow the flask to dry at 100° C. for 10 min. and remove any hydrochloric acid fumes with an air stream. Dissolve the dicarboxylic acid in 2 ml. of neutral, 95 per cent. alcohol, add 20 ml. of water, and warm to complete solution. Titrate, with phenolphthalein as indicator, with 0·02 N aqueous sodium hydroxide. One ml. of 0·02 N sodium hydroxide $\equiv$ 3·74 mg. of pyrethrin II. Carry out blank determinations on the reagents. These should not exceed 0·1 ml. of 0·01 M iodate and 0·25 ml. of 0·02 N sodium hydroxide.

DETERMINATION OF UNCOMBINED ACIDS (IF REQUIRED). Filter into a 500-ml. separating funnel the baryta extract containing the salts of the free acids, acidify with 5 ml. of dilute sulphuric acid, extract with light petroleum and continue the analysis as described above.

Application of the method to the determination of pyrethrins in light petroleum, ethylene dichloride, and acetone extracts of pyrethrum flowers gave comparable values for pyrethrin I in the extract with all three solvents, but the values for pyrethrin II in the acetone extract were high. Further tests showed that the method is applicable to the determination of pyrethrin I in oil solutions made from light petroleum, ethylene dichloride, or acetone extracts of the flowers. Determinations by two observers of the pyrethrin I content of commercial samples of pyrethrum extract in light and heavy mineral oils, and of the apparent pyrethrin I present in the form of uncombined acid, showed good agreement; the removal of uncombined acid before analysis is particularly important with solutions of high pyrethrin content. In a further series of experiments, values of the pyrethrin I content of oil solutions by the mercury-reduction method were slightly higher than those obtained by the steam-distillation method. E. M. P.

Water Analysis

Colorimetric Method of Determining the Hardness of Water. S. M. Dratschev (*Zavod. Lab.*, 1945, **11**, 46–48)—Tropaeolin OO, which gives difficultly-soluble, coloured compounds with magnesium and calcium salts, may be used for the colorimetric determination of water-hardness. Variation of pH within the range 6·5 to 8·7 does not affect the results. The method is sensitive to 0·5° of hardness and is accurate to 1 to 1·5°.

Preparation of standards for the scale of hardness—Mix at 20° C. equal volumes of (*a*) 1·23 g. of $MgSO_4.7H_2O$ dissolved in one litre of water, giving 28° of hardness, and (*b*) 0·369 g. of $CaSO_4.2H_2O$ dissolved in one litre of water, giving 12° of hardness, and dilute 10-, 20-, 30-, 40-, and 50-ml. portions of the mixture to 100 ml., thus obtaining solutions of hardness 2°, 4°, 6°, 8°, and 10°, respectively. In separate tubes place 1 ml. of distilled water and 1 ml. of each of the solutions, and to each add 0·5 ml. of tropaeolin OO solution (0·4 g. mixed with 50 ml. of alcohol until nearly dissolved and made up to 100 ml. with distilled water). This gives the lower part of the scale of hardness, *viz.*, 0°, 2°, 4°, 6°, 8°, and 10°. For the upper part of the scale, dilute 50-, 60-, 70-, 80-, 90-, and 100-ml. portions of the mixture above to 100 ml., to give solutions of hardness, 10°, 12°, 14°, 16°, 18°, and 20°, but take 1 ml. of each solution with 1 ml. of the tropaeolin OO solution.

Procedure—Pipette 1 ml. of the water to be tested into a tube similar to those used for the hardness scale, add 0·5 ml. of the indicator solution, mix, and compare the coloration with the lower set of standards. If the hardness exceeds 10°, add a further 0·5 ml. of indicator and compare with the upper scale. Wide variations in the ratio of magnesium and calcium salts in the water tested appear to have no effect on the results. G. S. S.

[The degrees of hardness in this abstract are in terms of CaO.]

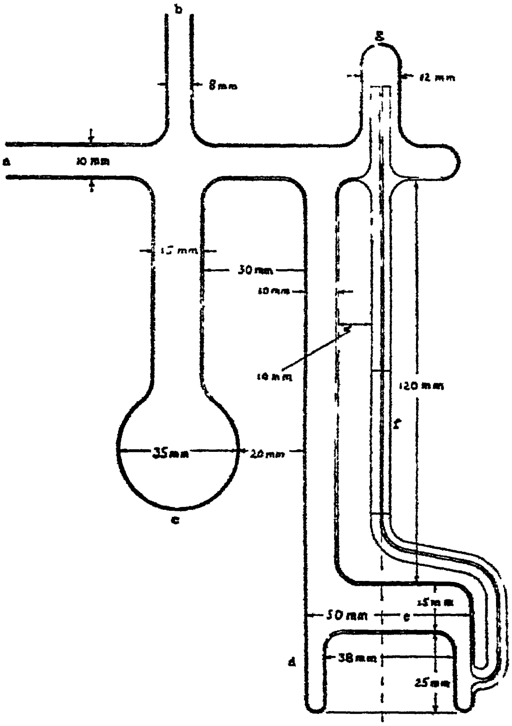
Physical Methods, Apparatus, etc.

New Type of Precision Capillarimeter. J. J. Jasper and K. D. Herrington (*J. Amer. Chem. Soc.*, 1946, **68**, 2142–2144)—The device described was designed for the accurate measurement of the surface tension of pure compounds prepared in a vacuum system and available in amounts of approximately 10 ml. Details of the instrument are shown in the figure. A horizontal arm *a* carries the liquid receiver *c*, the capillary chamber *d*, and the capillary tube *f*. Tube *b* is connected to the vacuum system and tube *a* is sealed. The capillary tube extends into the housing *g* to prevent liquid from flowing into it from above. The re-entrant bottom *e* of the capillary chamber reduces the quantity of liquid required to give a flat liquid surface and hastens the establishment of thermal equilibrium. As the axis of the capillary *f* coincides with that of the chamber *d* there is no need to revolve the cathetometer telescope when measuring the capillary rise.

Choose the capillary tube and the larger cylindrical tube for the liquid reservoir with great care. Prepare the capillary and determine the radius and constancy of its bore by the procedure of Harkins and Brown (*Ibid.*, 1919, **41**, 499). Test the walls of the chamber for distortion by viewing through the chamber, along its diameter, a scale placed beyond.

Procedure—Transfer the liquid from the vacuum system to *c* by immersing the latter in a freezing-bath. Seal off the arm *b* and transfer the liquid to *d* by tipping the instrument.

Values for the surface tension of several pure liquids were determined and were found to agree closely with the values given in the "International Critical Tables." B. A.



Reviews

F. Pregl, Quantitative Organische Mikroanalyse. Fünfte Auflage. Nach dem gegenwärtigen Stand der Forschung und Praxis bearbeitet von H. Roth. Pp. xii + 317. Vienna: Springer-Verlag. 1947. Price 32 Swiss francs.

The various editions of this work were outlined about eighteen months ago (Analyst, 1946, **71**, 199) on the occasion of the appearance of Dr. Grant's new English version. To this series must now be added another German edition written by Dr. Roth, the publication of which supplies information concerning war-time developments on the continent hitherto unavailable in this country. Former reviews of the recent German texts have complained of the neglect of non-German progress; because of the history of the last few years the time is inopportune to complain that the omission has not been rectified in the book now under review.

The current English and German editions are similar in that the methods described in detail are usually those originated by Pregl, while alternative procedures are mentioned only briefly. It is, however, interesting that the Parnas and Wagner distillation apparatus favoured by Pregl for Kjeldahl determinations is retained in the English book, but has been displaced in the German by a simpler assembly. Also, the Meixner and Kröcker method for the determination of mercury is replaced by an improvement due to Boëtius. It is curious that ebullioscopic molecular weight determinations, including that devised by Pregl, are omitted by Dr. Roth as being of no practical importance: these methods are to be found in the English text, which also has a brief review of general physical methods. It is not proposed to note all the differences in detail between the two versions of Pregl's book now available, but the following items appear only in the German edition. Oxygen is determined directly by a combustion method due to Unterzaucher, in which the organic material is "cracked" and the hot gasses passed over carbon to form carbon monoxide; the latter is oxidised by iodine pentoxide and the iodine liberated is titrated. Nitrogen is determined by Lacourt's hydrogenation method, which uses nickel and thoria as a catalyst; ammonia is formed and is determined acidimetrically. Zimmermann's method for sulphur determination consists of fusion of the material with potassium in a sealed tube, followed by evolution of hydrogen sulphide which is absorbed in cadmium acetate and determined iodometrically. Brief reviews are given of methods for the determination of selenium and antimony. An additional procedure for phosphorus depends upon the formation of the blue molybdenum complex. Descriptions are given of Furter, Bürger, and Baláž alkoxyl determinations. The section on melting-points is expanded to include mixtures and mixed melting-points; it is illustrated with photomicrographs. A useful list of solvents for cryoscopic molecular weight determinations is given, together with their uses and molecular melting-point depressions.

In spite of the differences noted above, Dr. Grant and Dr. Roth have produced very similar texts and it is probable that only specialists will wish to incur the expense of buying both. Neither of the books retains the highly personal and entertaining writing of Pregl, and the reviewer wonders whether the time has arrived when this pioneer's monopoly of organic micro-analysis has passed and future editions should describe in detail the more modern alternative procedures.

G. H. WYATT

MODERN CEREAL CHEMISTRY. By D. W. KENT-JONES, Ph.D., B.Sc., F.R.I.C. and A. J. AMOS, Ph.D., B.Sc., F.R.I.C. Fourth Edition. Pp. vii + 651. Liverpool: The Northern Publishing Co. Ltd. 1947. Price 50s. net.

The first edition of this book was published in 1924 as a modest little volume of 324 pages. The then reviewer (ANALYST, 1924, 49, 609) accepted the "Modern" part of the title but regarded "Cereal Chemistry" as being rather too wide. The second edition of 1927 contained 446 pages and was little more than an expanded reprint of the first; but the third edition of 1939, with 720 pages, was a real justification of the title. It became the standard work on flour and bread, and quotations from it were accepted as authoritative. Owing to the loss of unsold copies by enemy action, this edition was soon unobtainable and second-hand copies were at a premium.

Now an enlarged edition, the fourth, has appeared under joint authorship with Dr. Amos, the increase in material amounting to approximately 50 per cent. A change in set-up—a credit to the publishers and a delight to the reader—has resulted in fewer pages, achieved by about one-third more words per line and one-fifth more lines per page. The principal increases are in Wheats of the World, Other Cereals, Nutritive Value of Cereals, Rations for Livestock and Analytical Procedure. The only significant reduction is in Conditioning and the Effect of Heat on Wheat and Flour.

The arrangement follows previous issues—for which old and familiar users will be glad—and is particularly appropriate to the subject. Since the index of authors refers to paragraphs it would be helpful if each page quoted the paragraph number.

The material is really comprehensive and cannot be found collectively elsewhere. The wealth of data, the critical review and the summing-up of evidence are the principal features that characterise the volume and assure for it a prominent place in and out of the bookshelves of all concerned with cereal chemistry or with the production of food and food products from cereals. Apart from the mechanical testing of flour and dough, analytical procedures occupy nearly one-quarter of the space and special attention is given to vitamin assays including microbiological methods, to which the authors have materially contributed.

Knowing the authors, interest is naturally aroused in their approach to certain controversial matters. The reviewer of the first edition described it as "a plea for white flour and for the bleaching and 'improving' of white flour" and stated that "many of the views expressed and quoted will have to be revised." Such revision was made in subsequent editions and has been continued, through a more descriminating selection from the vast knowledge now available on nutrition, and the special pleading has become more of a judicial survey. The authors' views on giving the public what it wants have not changed; and although they may appear "bleached" and, indeed, "improved" by the narrowing down of the controversy from white flour *v.* wholemeal flour to enriched white flour *v.* near-white flour, the calories remain the same.

J. R. NICHOLLS

NOVEMBER, 1947 Vol. 72, No. 860

THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

An Ordinary Meeting of the Society was held on Wednesday, November 5th, 1947, at 6 p.m., in the Chemical Society's Rooms, Burlington House, London, W.1. The President, Mr. Lewis Eynon, was in the Chair. The following papers were read and discussed:—"The Determination of Arsenic Pentoxide in White Arsenic," by D. A. Lambie; "A Semi-micro Combustion Method for the Determination of Organic Carbon," by J. B. Rickson; "Some Observations on the Semi-micro Determination of Carbon and Hydrogen by the Sucharda and Bobranski Method, using a Macro-Balance," by Frank Goulden.

NEW MEMBERS

Frank John Allen, A.R.I.C.; Donald Wheeler Browne, B.Sc., Ph.D. (Lond.); Edwin Dawson Chilwell, B.Sc. (Lond.), A.R.I.C.; Gerald Harry Edwards, B.Sc. (Lond.), F.R.I.C.; Kenneth Sydney Fowler, B.Sc. (Reading), A.R.I.C.; Philip Harry Freestone, B.Sc. (Lond.), A.R.I.C.; Peter Fuller; Ralph Goulden, A.R.I.C.; Walter Hirsch, D.Phil.nat. (Frankfurt); Mervyn Henry Jenkins, B.Sc. (Pretoria); Mrs. Bertha Lamb, B.Sc. (Lond.); James Muil Leitch, B.Sc. (Glas.), A.R.I.C.; Douglas Breed Rogers; Leonard Seal, A.Met. (Sheff.); Mohamed El Shahat, M.Pharm., D.Biochem.; Herbert Timmington, B.Pharm., Ph.C.; John George Waller, B.Sc. (Lond.), A.R.I.C., A.R.C.S.

NORTH OF ENGLAND SECTIONS

A meeting of the Section was held at Manchester on Saturday, April 19th, 1947. The Chairman, Mr. C. H. Manley, presided over an attendance of thirty-six. The following papers were presented and discussed:—"The Determination of Organic Phosphorus," by C. H. Manley, M.A., F.R.I.C. and H. Lobley; "Ice Cream," by E. L. E. Humphries, A.R.I.C.

MICROCHEMISTRY AND PHYSICAL METHODS GROUPS

A joint meeting of the Physical Methods Group with the Microchemistry Group was held on Friday, September 26th, 1947, in the Chemical Lecture Theatre of the University Chemical Laboratories, Cambridge. The chair was occupied by Mr. R. C. Chirnside and the attendance numbered eighty. The following papers were presented and discussed:—"Micro-methods of Molecular Weight Determination," by Cecil L. Wilson, M.Sc., Ph.D., F.R.I.C.; "Turbidimetric Methods used in Agricultural Analyses," by J. Tinsley, B.Sc., Ph.D., F.R.I.C.; "Microchemical Applications of Potentiometry," by J. T. Stock, M.Sc., F.R.I.C.; "Micro-Analyses using X-ray Diffraction Technique," by H. P. Rooksby, B.Sc., F.Inst.P.

The meeting was preceded by visits to the Colloid Science Department and the Radio-Chemistry Laboratory of the University.

Polarographic Discussion Panel of the Physical Methods Group—Meetings of the Panel were held on July 25th, at University College, London, and on October 3rd, at Norwood Technical College. The discussion at the former meeting was opened by Professor J. Heyrovský and Dr. R. Brdička, and that at the latter by Mr. A. S. Nickelson, Mr. L. Airey and Dr. F. J. Bryant.

The Direct Colorimetric Determination of Tungsten in Cast Iron

By W. WESTWOOD AND A. MAYER

A NUMBER of gravimetric procedures have been used by various authors; these include precipitation of tungstic oxide by means of nitric acid, cinchonine,[1] rhodamine B,[2] tannin-phenazone,[3] tannin-antipyrene,[4] benzidine[4] and 8-hydroxyquinoline.[4,5] All these methods have the disadvantage that with small amounts of tungsten precipitation is incomplete; also the final product is usually contaminated by small amounts of various elements such as iron, molybdenum, chromium, vanadium, silicon, titanium and zirconium, which necessitate its purification. Moreover, the conversion factor of tungstic oxide to tungsten, 0·7931, is very high. A volumetric method has been described,[6] in which reduction by means of lead amalgam is followed by titration with permanganate; but this method also requires complete preliminary separation of tungsten.

Several colorimetric methods are available in which reduction of tungstic oxide with stannous chloride[7] or titanium trichloride[8] yields blue colloidal lower oxides, but the sensitivity of these reactions is not very great. Dithiol[9] gives with tungstate a blue colloidal precipitate, but molybdenum interferes by forming a green precipitate. A method depending on formation of the dithiol - tungsten complex at elevated temperature and its extraction with amyl acetate has been described recently[10]; molybdenum must first be removed by a similar procedure carried out at room temperature. A rough colorimetric method makes use of the violet colour given by a tungstate with rhodamine B in dilute hydrochloric acid.[11] Hydroquinone[12,13,14] in concentrated sulphuric acid has been used extensively as a colorimetric reagent for tungsten. This reaction is interfered with by titanium, which also yields a brown colour. The two colours can be differentiated by measurement of the absorption at different wavelengths, but the method is tedious and requires separation of the tungsten from iron. Niobium, moreover, behaves much like tungsten in this reaction.

The yellow or green colour formed by thiocyanate with tungsten in presence of a reducing agent has been the subject of much study,[15,16,17,18,19,20,21] and various conditions have been prescribed for the reaction. We have previously used this reaction as the basis of a method for determining tungsten in cast iron.[18] The necessity for preliminary separation from iron and other elements, which led to low results when the tungsten present was less than 0·5 per cent., besides other minor disadvantages, rendered it not wholly satisfactory. An attempt to improve the method by extracting the colour (produced by adding sodium thiocyanate, stannous chloride and hydrochloric acid to a standard tungstate solution and then heating on a water-bath) by means of organic solvents such as ether, *cyclo*hexanol, butyl acetate or, better, amyl acetate, was partially effective. Variations in the intensity and rate of the colour development consequent on small changes in the concentration of reagents and the period of heating made it difficult to obtain reproducible results. In the course of these experiments it was noticed that heating was not necessary when the hydrochloric acid content was more than 70 per cent. of the concentrated acid by volume and that the yellow colour was more stable when it was formed in the cold. For example, a solution containing 0·4 mg. of tungsten as sodium tungstate, prepared by dissolving 0·1261 g. of pure dry tungstic oxide, WO_3, in a little sodium hydroxide and diluting to a litre (1 ml. $\equiv$ 0·1 mg. of tungsten) was placed in a beaker; to this were added 6 ml. of water, 1·5 ml. of sodium thiocyanate solution (500 g. per litre), 5 ml. of stannous chloride solution (200 g. in 1 litre of hydrochloric acid), and 15 ml. of hydrochloric acid. A yellow colour developed on standing. It was measured at intervals on a Spekker absorptiometer, using a 2 cm. cell and Ilford violet filters No. 601. The colour reached its maximum intensity in 17 minutes and remained stable for at least an hour. This result was sufficiently satisfactory to warrant a full investigation to establish the optimum conditions.

EXPERIMENTAL

Influence of thiocyanate concentration on the intensity of the colour—Different amounts of sodium thiocyanate solution (500 g. per litre) ranging from 0·5 to 3·5 ml. were added to a standard tungsten solution together with the reagents used in the preliminary experiment above. With each experiment a "blank" without tungsten was carried out. The results (see Table I) show that the "blanks" developed a colour (brown) when more than 2 ml. of thiocyanate solution were present, and that the optimum amount of thiocyanate solution was 1·0 to 1·5 ml., *i.e.*, 0·75 g. to 1·25 g. of sodium thiocyanate, in 50 ml. of solution.

TABLE I

INFLUENCE OF THIOCYANATE CONCENTRATION

$NaCNS$ solution ml.	Standard tungsten solution ml.	Drum reading
0·5	2·0	0·73
0·5	nil	1·00
1·0	2·0	0·545
1·0	nil	0·995
1·5	2·0	0·535
1·5	nil	0·995
2·0	2·0	0·515
2·0	nil	0·97
2·5	2·0	0·50
2·5	nil	0·95
3·5	2·0	0·435
3·5	nil	0·89

Influence of hydrochloric acid concentration on the colour—It was known from previous observations that variation of the hydrochloric acid concentration had pronounced effects on the intensity of the colour. This effect was investigated in detail as follows. To a series of solutions of similar tungsten content were added 1·5 ml. of the thiocyanate solution, and 20 ml. of hydrochloric acid of varying concentration, followed by 5 ml. of the stannous chloride solution. The total volume was kept constant throughout. After standing for 17 minutes the colours were measured on the Spekker absorptiometer in the usual manner. The results, given in Table II, show that the optimum concentration of hydrochloric acid lies between 70 and 80 per cent. of the concentrated acid in the final reaction mixture.

TABLE II

INFLUENCE OF HYDROCHLORIC ACID CONCENTRATION

Hydrochloric acid, per cent. v/v of conc. acid	Appearance of solution	Drum reading
16	Colourless	0·99
30	Colourless	0·98
40	Very slightly yellow	0·97
50	Yellow	0·88
60	Yellow	0·86
70	Yellow	0·60
80	Yellow	0·59
90	Precipitate (probably $NaCl$)	0·615 (after filtration)

In subsequent experiments the reaction solutions were always diluted to a standard volume, 50 ml.

Influence of concentration of stannous chloride reagent on the colour—This was investigated by taking 3 ml. of the standard tungsten solution, diluting to 10 ml. with water, adding 1·5 ml. of the thiocyanate solution and varying amounts (2 to 12 ml.) of stannous chloride reagent (250 g. in 1 litre of hydrochloric acid) and diluting to 50 ml. with hydrochloric acid. This gave an acidity of 77·5 per cent. in terms of concentrated hydrochloric acid. The intensity of the colour increased with increasing amounts of stannous chloride solution up to 9 ml. Further additions up to 12 ml. had no further effect.

For subsequent experiments the stannous chloride and hydrochloric acid were combined in a single reagent. The new stannous chloride reagent contained 70 g. of stannous chloride in 1 litre of hydrochloric acid.

Development of the colour with time—This was investigated by observing the drum reading at regular intervals for two solutions of tungsten, one containing 0·1 mg. and the other 0·6 mg. The curves obtained are shown in Fig. 1. Development of the colour, both for large and small amounts of tungsten, is complete within 17 minutes and then remains constant for at least 2 hours.

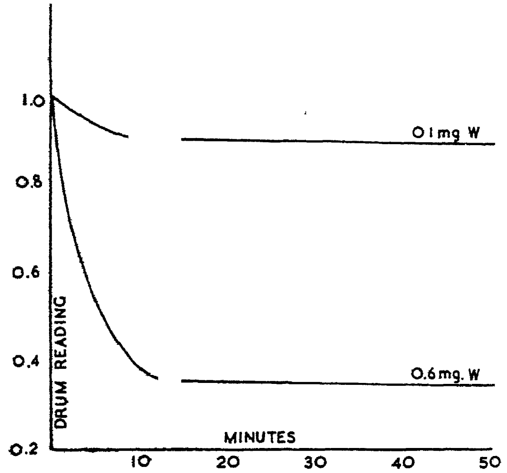


Fig. 1. Development of colour with time.

CALIBRATION GRAPH—A preliminary calibration graph was constructed based on various amounts of standard tungsten solution made up to 10 ml. with water, to which were added 1·5 ml. of sodium thiocyanate solution (500 g. per litre) and sufficient stannous chloride reagent (70 g. in 1 litre of concentrated hydrochloric acid) to make 50 ml. in a graduated flask. The colour was allowed to develop for 20 minutes and the absorption measured on the Spekker absorptiometer, using 2 cm. cells and violet filters No. 601. The graph was a straight line and entirely reproducible. It conformed to the equation

$$\text{Tungsten per cent.} = (0{\cdot}96 - \text{drum reading})/1{\cdot}103$$

The colours were stable for at least 2 hours. The range of tungsten that could be determined under these conditions was 0 to 0·87 mg.

From the investigation described above it appeared possible that the method could be adapted readily to the direct determination of tungsten in cast iron. For this purpose the effects of various alloying elements and other ions were investigated. To prevent precipitation of tungstic oxide during dissolution of the sample in acid, it was advisable to have tartaric acid present, which would form a complex with tungstate. The tartrate, when tried on pure solutions, did not interfere with the colour development.

EFFECT OF ALLOYING ELEMENTS AND OTHER IONS—

Iron—Iron is without effect. A calibration graph was prepared under the same conditions as before (above), except that the solutions contained, also, 0·1 g. of iron as ferric chloride and 1 g. of tartaric acid, added before developing the colour. The iron underwent reduction immediately and the graph was found to be identical with that for a pure tungsten solution.

Molybdenum—Solutions of molybdate were added to a synthetic sample solution containing 0·1 g. of iron, 1 g. of tartaric acid, and the usual reagents. On addition of the stannous chloride, the deep red ferric thiocyanate colour disappeared immediately, whereas the amber-coloured molybdenum thiocyanate persisted for about a minute and then faded rapidly. At the end of 20 minutes the colours had faded almost completely to a faint yellow. The drum difference readings for quantities of molybdenum corresponding to 0·5, 1·0, 2·0, 2·5, and 3·0 per cent. on a 0·1-g. sample of iron were 0·005, 0·01, 0·02, 0·025, and 0·03 respectively. These readings showed that amounts of molybdenum normally present in cast iron did not interfere; larger amounts could be allowed for by using a correction graph (1 per cent. of Mo ≡ 0·009 per cent. of W). The persistence of the amber colour of the molybdenum complex for a few minutes serves to indicate the presence of molybdenum.

Other cations—The possible interference of a number of elements was investigated by adding them to tungsten-free solutions containing 0·1 g. of iron and treating with the usual reagents (see Table III). No interference was encountered from aluminium, antimony, bismuth, lead, manganese, titanium or zirconium in the maximum amounts normally present in cast iron. Chromium, large amounts of cobalt, nickel and vanadium interfered by the formation of coloured ions. Arsenic, selenium and tellurium were reduced to the elements, giving brown colours or precipitates. Copper formed a precipitate of cuprous thiocyanate.

Chromium, cobalt, nickel, tellurium and vanadium can be corrected for by a differential method. This involves measurement of the colour of the final solution containing all reagents except the thiocyanate as described in the procedure (p. 468).

It was found that arsenic below 0·05 per cent. and copper below 0·5 per cent. did not interfere.

Selenium, if present, and copper and arsenic in excess of the amounts mentioned must be removed.

Anions—Phosphate (≡ 2 per cent. of P) and sulphate did not interfere. In presence of fluoride or nitrate, colour development was almost completely inhibited.

TABLE III

EFFECTS OF VARIOUS ELEMENTS

Element	Quantity taken, as per cent. on a 0·1-g. sample of iron	Observation	Drum reading
Manganese	2·2	Colourless	1·00
Aluminium	6·0	Colourless	1·00
Titanium	1·0	Colourless	1·00
Vanadium	1·0	Yellow	0·79
Nickel	10·0	Very slight green	0·955
Lead	0·5	Colourless	1·00
Zirconium	1·5	Colourless	1·00
Bismuth	0·5	Colourless	1·00
Arsenic	0·4	Brown precipitate	0·44
Antimony	1·5	Colourless	1·00
Cobalt	1·0	Very slight green	0·975
Copper	4·0	White precipitate	Too dense to read
Chromium	4·0	Greenish	0·90
Selenium	0·2	Orange turbidity	0·86
Tellurium	0·2	Brown colour	0·92

DEVELOPMENT OF COLOUR WITH TIME—This had been investigated previously, with pure solutions, but it was necessary to find out whether the rate of colour development was affected by the presence of tartrate and iron. The results obtained with a solution containing 0·1 g. of iron, 1 g. of tartaric acid, 0·8 mg. of tungsten as sodium tungstate and the usual reagents, are shown in Table IV.

TABLE IV

RATE OF DEVELOPMENT OF THE COLOUR

Time in minutes	Drum reading	Apparent tungsten present, mg.
0	1·00	0·00
2	0·46	0·46
2·5	0·42	0·49
3·5	0·355	0·55
4·5	0·315	0·585
5·5	0·275	0·62
6·5	0·24	0·655
7·5	0·218	0·675
8·5	0·20	0·690
9·5	0·17	0·715
11·5	0·15	0·735
12·5	0·135	0·745
16·5	0·09	0·785
20·0	0·075	0·80
30·0	0·075	0·80

The presence of other ions had very little effect on the rate of development of the colour with time. Twenty minutes is the time necessary for complete colour development.

TEMPERATURE—The effect of the temperature of the solution at the time of colour development was investigated by adding the usual reagents to a standard tungsten solution and heating the hydrochloric acid before addition. It was found that the rate of development of the stable colour was not affected by variations of temperature between 15° and 40° C.

It appeared, however, that the intensity of the final colour varied slightly with the temperature of the stannous chloride - hydrochloric acid reagent. It was advisable to adjust the temperature of the reagent to within ±2° C. of a convenient temperature, say 20° C.

APPLICATION TO CAST IRON—

The method was next applied to samples of tungsten-free iron to which increasing amounts of standard tungsten solution were added. One g. of sample plus tungsten solution was dissolved in hydrochloric acid in presence of tartaric acid, filtered through a pad and diluted to 100 ml. A 10-ml. portion, equivalent to 0·1 g. of sample, was placed in a 50-ml. graduated flask. To this was added thiocyanate solution and stannous chloride reagent, and after standing for 20 minutes the colours were measured on the Spekker absorptiometer in the usual manner. Another 10-ml. fraction was treated similarly but the thiocyanate was replaced by 1·5 ml. of water. The difference between the two readings was plotted against the percentage of added tungsten. The graph was identical with that obtained previously. A number of samples of cast iron containing tungsten as an alloying element were then treated by the above procedure. The residues obtained after the first filtration were ignited, treated with hydrofluoric acid to remove silica, fused with sodium carbonate and extracted in hydrochloric - tartaric acid mixture. After making up to 100 ml. a 10-ml. fraction was taken and the tungsten colour developed in the manner described previously. The samples thus treated contained some tungsten in the acid-insoluble portion. The major part of the tungsten appeared to dissolve readily under the given conditions, and it is thought likely that only the tungsten present as carbide did not dissolve. The method, therefore, had to be modified, so as to include the insoluble portion of the tungsten. This was done as indicated above, but the extract was added to the main filtrate before making up to 100 ml.

The proposed method for the determination of tungsten in cast iron is as follows.

Method

Reagents—*Diluted hydrochloric acid* (1 + 4). *Tartaric acid solution*, 500 g. per litre. *Tartaric acid solution*, 20 g. per litre. *Hydrogen peroxide*, 20 vol. *Sodium thiocyanate solution*, 500 g. per litre. *Stannous chloride solution*, 70 g. dissolved in 1 litre of hydrochloric acid. *Sodium carbonate*, anhydrous. *Hydrofluoric acid*. *Nitric acid*.

Procedure—Dissolve 1 g. of sample in 30 ml. of diluted hydrochloric acid (1 + 4) and 20 ml. of tartaric acid solution (containing 500 g. per litre). When dissolution is complete add 15 ml. of hydrogen peroxide, boil for 5 minutes, filter through a pad, collecting the filtrate in a beaker, and wash the pad with hot tartaric acid solution containing 20 g. per litre. Reserve the filtrate. Transfer the entire residue to a platinum crucible and ignite at a temperature of less than 750° C. Treat the ignited residue with 2 drops of nitric acid and 5 to 10 drops of hydrofluoric acid and heat to remove silica. Again ignite at less than 750° C. for a few minutes and then fuse the residue with about 1 g. of sodium carbonate. Cool the melt and extract it in the reserved filtrate. Cool and dilute this to 100 ml. in a graduated flask. Pipette a 10-ml. fraction into a 50-ml. graduated flask and add to it 1·5 ml. of sodium thiocyanate solution from a burette. Dilute the solution to 50 ml. with stannous chloride reagent adjusted to 20° C. ± 2° C. Prepare a blank solution by taking another 10-ml. fraction, adding to it 1·5 ml. of water and stannous chloride reagent to make up 50 ml. Allow both solutions to stand for 20 minutes and then measure the colours on the Spekker absorptiometer, using 2-cm. cells, Ilford violet filters No. 601, and setting water/water 1·00. The difference of the two readings is a measure of the tungsten content of the sample.

Standardisation—Standard tungstate solution—Dissolve 1·2610 g. of pure, dry tungstic oxide, WO_3, in 10 ml. of sodium hydroxide solution (containing 10 g. per litre) and dilute to 1 litre. One ml. of this solution is equivalent to 1 mg. of tungsten, *i.e.*, to 0·1-per cent. on a 1-g. sample. Add increasing volumes of this solution to 1-g. samples of tungsten-free cast iron and carry out the normal procedure. Plot the difference drum readings against percentage of tungsten added, to obtain the calibration graph.

In the authors' laboratory this conformed to the equation

$$\text{Tungsten per cent.} = 0{\cdot}906 \times \text{drum reading difference.}$$

Notes—(1) The method is applicable to all cast iron samples except those containing copper in excess of 0·50 per cent., arsenic in excess of 0·05 per cent. or selenium.

(2) The procedure described gives a graph that covers a range of tungsten from 0 to 0·85 per cent. For amounts in excess of that, a smaller fraction than 10 ml. must be taken and made up to 10 ml. with water; alternatively, a smaller weight of sample may be used.

(3) The stannous chloride reagent should be prepared fresh daily.

(4) Sodium thiocyanate cannot be replaced by potassium thiocyanate because [illegible] latter would result in the formation of insoluble potassium tartrate.

(5) Samples that will not dissolve under the conditions described may be treated as follows. Dissolve 1 g. of sample in 30 ml. of diluted hydrochloric acid (1 + 1) and when

dissolution is complete oxidise by addition of nitric acid. Evaporate to dryness, and bake to dehydrate silica and remove nitric acid. Cool, add 10 ml. of hydrochloric acid and digest to dissolve salts. Add 20 ml. of tartaric acid containing 500 g. per litre, heat to boiling and filter. Continue as described in the procedure. In this case most of the tungsten will be in the residue.

The method occupies 1½ hours and is accurate to ±0·01 per cent.

SUMMARY—A method is proposed for determining small amounts of tungsten in cast iron. It depends upon the formation of an intensely yellow-coloured tungsten thiocyanate complex in strong hydrochloric acid solution in presence of stannous chloride. The colour is measured on the Spekker absorptiometer. Alloying elements in amounts normally present in cast iron do not interfere. The method is speedy and has been found to yield accurate and reproducible results.

The authors wish to express their thanks to the Director and Council of the British Cast Iron Research Association for permission to publish this work.

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THE BRITISH CAST IRON RESEARCH ASSOCIATION
ALVECHURCH, BIRMINGHAM

January, 1947

The Determination of Mercury by means of Dithizone

BY H. BARNES

THE following notes, based on the experience of several years' use of dithizone for routine and other determinations, are prompted by the paper of Milton and Hoskins[12] on the estimation of mercury by a modification of the method of Reith and van Dijk.[13] It is not intended in any way to criticise their paper, but rather to amplify certain of the points raised with a view to assisting other users of this reagent. Further details of the technique used and an account of the separation of mercury from copper by a simple dithizone method involving the use of potassium cobalticyanide, have already been given by Barnes.[1,2]

The routine work has been largely concerned with the determination of the rate of release of mercury from anti-fouling compositions into sea water, and in these routine determinations 100 ml. of solution containing from 1 to 5 μg. of mercury are extracted with 10 ml. of a chloroform solution of dithizone of approximate strength 20 mg. per litre.[6] Dithizone has also been used for determining mercury in small paint samples when 100 ml. of solution, adjusted to contain approximately 100 μg. of mercury, are extracted with 50 ml. of a chloroform solution of dithizone of the above strength. Subsidiary work has been carried out on the estimation of traces of copper by this reagent.

A mixed colour method has always been employed and a Spekker absorptiometer, with a 1-cm. cell and blue-green filters (Ilford 603).

(1) *Purification and storage of dithizone solutions*—The precipitation of dithizone (0·5 g.) from the ammoniacal extracts is always carried out by addition of a strong solution of sulphur dioxide. The latter is added to the combined ammoniacal extracts (after separation of impurities as described by Milton and Hoskins[12]) contained in a litre separating funnel, and the precipitated dithizone is shaken out with several small additions of chloroform (or carbon tetrachloride), the extract being run off each time into a 100-ml. volumetric flask used only for storing this reagent. The combined extracts are made up to the mark with the solvent used, covered with a small quantity of the strong sulphur dioxide solution (although Sandell[15] states that this procedure does not increase the stability of the solution) and stored in a refrigerator. Appropriate quantities are taken from this stock and diluted with the organic solvent just before use. The purified dithizone is never stored as the dry product after removal of the solvent. Hydrochloric acid is not used to precipitate the dithizone from the ammoniacal solution, for there is some evidence that, on occasions, it leads to erratic results in subsequent determinations.

(2) *Working conditions*—To obtain the best results with the Spekker absorptiometer it has been found necessary to use it in a dark room. Further, when using dilute dithizone solutions, the extraction of mercury is always carried out in a darkened room and the reading of transmittances taken as soon as possible after extraction (see, for example, Laug and Nelson[10]). It has never been found necessary to treat the dithizone extract with a dehydrating agent. A small roll of filter paper is inserted into the stem of the funnel and the extract run off slowly into the absorptiometer cell. The removal of excess dithizone by ammonia has been stated by Leibhafsky and Winslow[11] to be less satisfactory than a mixed colour method. There is a danger that with the more alkaline solutions some of the metal complex may be decomposed and under less alkaline conditions the dithizone may not be completely extracted. Further, the extra manipulation involved enhances the possibility of decomposition of the unstable mercury complex. In general, dithizonates are, however, more easily decomposed in carbon tetrachloride solutions. Excess dithizone is more easily removed from carbon tetrachloride solutions than from chloroform solutions, since the partition coefficient $\mathrm{Dz}_{\mathrm{water}}/\mathrm{Dz}_{\mathrm{organic\ solvent}}$ is greater for the former solvent at the *p*H values normally used (Clifford and Wichmann[4]). When an absorptiometer is available a mixed colour method would, in general, seem preferable.

(3) *Digestion*—If only small quantities of organic material are present, as in small paint samples, digestion with sulphuric acid followed by hydrogen peroxide (100 vol. M.A.R.) is satisfactory. After removal of excess of the latter with potassium permanganate the solution is treated with hydroxylamine hydrochloride and a direct mercury determination carried out on the solution thus obtained.

(4) *Extraction of copper and mercury by dithizone*—In their work, Milton and Hoskins[12] used chloroform solutions of dithizone; the marked contrast in the behaviour of chloroform and carbon tetrachloride solutions of the reagent with respect to the extraction of copper is to be stressed, since any method of separation of the two metals based on the relative rates of extraction is considerably affected by the solvent used. At low *p*H values the rate of extraction of copper by chloroform solutions of the reagent is very much slower than by a carbon tetrachloride solution. Thus, shaking a solution (100 ml.) containing 70 µg. of copper and 5·5 µg. of mercury at *p*H 1·2 for 1 minute with a chloroform solution of the reagent will only cause an error of 0·3 µg. in the mercury estimation. Quite large quantities of copper can be shaken with comparatively little extraction if the *p*H is kept low, as is indicated by Table I.

TABLE I

100 ML. OF SOLUTION; 10 ML. OF DITHIZONE IN CHLOROFORM; *p*H 1·4

"Spekker" set at 0·740 against pure chloroform.

Time of shaking, min.	0·6 mg. of copper Spekker reading (corrected)	1·5 mg. of copper. Spekker reading (corrected)
1	0·706	0·646
2	0·671	0·589
4	0·641	0·523
8	0·526	0·374

At higher *p*H values the extraction is more rapid (Table II), although still much slower than the extraction of mercury.

TABLE II

100 ML. OF SOLUTION; 10 ML. OF DITHIZONE IN CHLOROFORM; *p*H 4·2

"Spekker" set at 0·740 against pure chloroform.

Time of shaking, min.	7·5 μg copper Spekker reading (corrected)
1	0·660
2	0·587
4	0·517
10	0·506
15	0·500

The figures in Table III illustrate the rapidity of extraction of mercury even at low *p*H values.

TABLE III

100 ML. OF SOLUTION; 10 ML. OF DITHIZONE IN CHLOROFORM; *p*H 1·4

"Spekker" set at 0·740 against pure chloroform.

Time of shaking, min.	5·5 μg. of mercury Spekker reading (corrected)
1	0·603
2	0·608
4	0·605
10	0·608

In contrast, even at low *p*H values (Table IV), the copper is rapidly extracted by a carbon tetrachloride solution of the reagent and at a higher *p*H values still more rapidly.

TABLE IV

100 ML. OF SOLUTION; 10 ML. OF DITHIZONE IN CARBON TETRACHLORIDE; *p*H 1·16

"Spekker" set at 0·740 against pure carbon tetrachloride.

Time of shaking, min	5·2 μg of copper Spekker reading (corrected)	2·6 μg. of copper Spekker reading (corrected)
1	0·630	0·705
2	0·616	0·670
5	0·598	0·671

With certain metals, including bivalent copper and mercury, dithizone combines to form keto- and enol-metal derivatives of the type XDz_2 and XDz, respectively. The former are obtained in acid or neutral solutions and the latter in basic solutions or with a large deficiency of dithizone. The relations are clearly complex and there are few data on which to base theoretical deductions, although a simple case in which equilibrium was established has been treated in some detail by Kolthoff and Sandell,[9] (see also Wichmann[17]). Even less is known about the rates of the reactions concerned than the equilibrium constants. (For recent work on the formation of metal complexes, see Calvin and Wilson.[3]) The marked effect of *p*H may be taken to indicate that the reaction between metal and dithizone takes place in the aqueous phase, and it may be assumed that partition equilibrium between the aqueous and organic solvent phases is rapidly established on vigorous shaking for both dithizone and metal dithizonates. The rate at which metal dithizonate is brought into the organic solvent will therefore depend on the rate of production of the appropriate form of dithizone in the aqueous phase and the rate of reaction with the metallic ion concerned.

From the analytical standpoint, in the separation of copper and mercury the relative rates of transference may be of more practical importance than the equilibrium values, for the latter may not be attained under normal working conditions: this is particularly so when working at low *p*H values with chloroform solutions of the reagent.

Greenleaf[5] states that copper is readily extracted by a carbon tetrachloride solution of dithizone at *p*H 2, and Sandell[14] that it is completely extracted by 30 to 45 seconds vigorous shaking with the same reagent (3 to 20 μg. of copper in 25 ml. of sample). However, the latter investigator, using an extraction technique and a working curve based on pure copper solutions, found an incomplete recovery of added copper. The same author, in a later publication,[15] recommends shaking the solution to be extracted for 2 minutes. Sherman and

McHargue[16] point out that extraction of copper as well as zinc proceeds more slowly than is usually stated in the literature, whilst the slow rate of extraction in acid solution and the greater ease of extraction of zinc with carbon tetrachloride solutions has been noted by Hibbard.[7,8]

This work was carried out by the author when Investigator to the Marine Corrosion Sub-Committee of the Iron and Steel Institute.

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THE MARINE STATION
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Determination of Small Quantities of Manganese in Caustic Soda

BY C. G. ETHRINGTON AND J. W. HUGHES

A METHOD was required for the estimation of quantities of manganese of the order of 0·03 parts per million, in the routine examination of commercial caustic soda samples.

The most commonly known methods for the estimation of manganese have been those utilising bismuthate or persulphate,[1,2] though four reagents are described by Vogel,[3] who refers to potassium persulphate as widely used but unsatisfactory owing to the incompleteness of reaction, and potassium periodate as the most satisfactory but subject to limitations in respect of manganese, chlorine and iron contents. Two methods[4] have been suggested recently for the estimation of manganese in caustic soda solutions, but they were not considered suitable for rapid accurate routine tests; a later method[5] was developed, but was not applicable to our particular requirements owing to the concentration of salts that would be present after the necessary neutralisation of the sodium hydroxide.

In the past, use has been made of flocculent precipitates for collecting small amounts of finely dispersed metals in solutions.[6] Attempts were therefore made to separate small quantities of manganese from caustic soda solutions by co-precipitation with magnesium hydroxide and to estimate their amount by a persulphate or periodate method.

This method was tried on 300-ml. portions of a 20 per cent. sodium hydroxide solution, to which had been added known amounts of a standard solution of manganous sulphate (1 ml. $\equiv 3{\cdot}078 \times 10^{-6}$ g. of Mn). The manganese was then "extracted" as follows. To 15 ml. of a 10 per cent. solution of $MgSO_4.7H_2O$ was added a little of the sodium hydroxide solution, the mixture was shaken to a thin cream, and then the remainder of the 300 ml. was added, and the mixture poured into a cylinder, with maximum stirring effect, to ensure distribution of the magnesium hydroxide; after standing overnight at room temperature, the supernatant liquor was syphoned off, and the residual "mud" was taken as containing the magnesium and manganese.

The first attempts to estimate the manganese in the mud were made with ammonium persulphate. Several variations on the method were applied,[1,3,7,8] but this reagent was later regarded as unsatisfactory because the results were not always reproducible, the permanganic colour being unstable and often affected by a brown tint; moreover, the manipulation was lengthy, owing mainly to the necessity for removal of chlorides as silver chloride, by filtration, Nevertheless, there was every indication that the full manganese content of the soda samples was being removed by the precipitation of the magnesium. The use of persulphate was discontinued and the periodate method was developed in conjunction with the collection of the manganese on precipitated magnesium hydroxide.

The preparation of the sodium hydroxide solutions and the precipitation and settling of the magnesium hydroxide were carried out as before. The supernatant liquor was siphoned off, leaving a total volume of "mud" and clear liquor of 40 ml. This was transferred to a 200-ml. tall-form pyrex beaker and to it was added 0·1 g. of $Na_2SO_3.7H_2O$, followed by 20 ml. of diluted sulphuric acid (1 + 1). The acidified solution was boiled for 15 minutes to remove all traces of sulphur dioxide, which would produce a brown colour on the subsequent addition of periodate. Five ml. of syrupy phosphoric acid were next added and then 0·1 g. of potassium periodate and the solution was boiled for 15 minutes. (The colour was slow to develop and it was necessary to boil for at least 5 minutes after the appearance of a pink coloration.) The solution was transferred to a Nessler glass and matched after 5 minutes against 0·001 *N* potassium permanganate in a second Nessler glass containing 5 per cent. phosphoric acid solution which had been treated with periodate. The 0·001 *N* permanganate was freshly prepared from 0·1 *N* solution. A blank determination on caustic soda without addition of manganese was made with each test, in order to allow for the manganese content of the reagents. Results are shown in Table I.

TABLE I

Std. $MnSO_4$ soln. added, ml.	Equivalent Mn, g $\times 10^{-5}$	0·001 *N* $KMnO_4$, ml.			Equivalent Mn, g. $\times 10^{-5}$	Error %
		Total	Blank	Nett		
6 0	1·85	2·60	0·80	1·80	1·98	+ 7·0
6·0	1·85	2·60	0·80	1·80	1·98	+ 7·0
6·0	1·85	2·85	1·20	1·65	1·81	− 2·2
6·0	1·85	2·85	1 20	1·65	1·81	− 2·2
7·0	2·15	3·00	1·00	2·00	2·20	+ 2·3
7·0	2·15	3·10	1·00	2·10	2·31	+ 7·4

In this series of tests no difficulty was experienced in matching the colours with 0·001 *N* permanganate.

Working on the assumption that the periodate method as outlined above was reasonably satisfactory, magnesium sulphate was added to sodium hydroxide solutions of various concentrations in order to determine suitable conditions for rapid settling; it was decided that a strength of approximately 10 per cent. of sodium hydroxide would give good settling of the magnesium hydroxide in 1 hour at room temperature, without involving undue dilution and increase in bulk.

The complete procedure adopted for the collection and estimation of manganese in caustic soda samples is as follows.

METHOD

Dilute the appropriate quantity of the caustic soda sample to about 10 per cent. concentration with distilled water. Put 15 ml. of a 10 per cent. solution of $MgSO_4.7H_2O$ in a tall cylinder (*e.g.*, of 500-ml. capacity, but dependent on the volume of sample solution required for a satisfactory estimation), add a little of the 10 per cent. sample solution, mix to a cream and then add the whole of the remaining sample solution, pouring from one cylinder to another to ensure good distribution of the magnesium hydroxide. Allow to settle for 1 hour and then siphon off the supernatant liquor, leaving 45 to 50 ml. of "mud" and liquor. Add to this 0·1 g. of $Na_2SO_3.7H_2O$ and 25 ml. of diluted phosphoric acid (1 + 1). Transfer the resulting clear solution to a 200-ml. tall beaker, rinsing the cylinder with water to give a final volume of about 120 ml. in the beaker, and cover this with a watch-glass. Boil the solution for 10 minutes to expel sulphur dioxide, add 0·4 g. of potassium periodate and boil for a further 10 minutes, adding water if necessary to maintain a volume of about 100 ml. Transfer the solution to a Nessler glass and after cooling for half an hour match it against standardised 0·001 *N* potassium permanganate by adding this to a Nessler glass containing 25 ml. of diluted phosphoric acid (1 + 1), 100 ml. of water and 0·4 g. of potassium periodate, previously boiled for 15 minutes.

Standardisation of the 0·001 *N potassium permanganate solution*—(i) Treat 6 ml. of standard manganous sulphate solution (1 ml. $\equiv 3{\cdot}078 \times 10^{-6}$ g. of Mn) with 0·1 g. of $Na_2SO_3.7H_2O$, add 100 ml. of water and 10 ml. of diluted phosphoric acid (1 + 1), cover with a watch-glass, and boil vigorously for 10 minutes. Then add 0·4 g. of potassium periodate and boil for 15 minutes, maintaining the volume at about 100 ml. Pour the solution into a Nessler glass (i).

(ii) Boil 10 ml. of diluted phosphoric acid (1 + 1), 100 ml. of water, and 0·4 g. of potassium periodate for 15 minutes, maintaining the volume at about 100 ml., and then pour into a Nessler glass. After half an hour add to this Nessler glass 0·001 *N* potassium permanganate sufficient to match the colour in Nessler glass (i).

In making the tests shown in Table II it was found that 1 ml. of permanganate solution (approximately 0·001 *N*) was equivalent to 1·024 × 10^{-5} g. of manganese.

No manganese was detected in the supernatant liquor from the samples containing up to 6 ml. of standard manganous sulphate solution. With 7 ml. and over, traces of manganese were found to be present, but in quantities too small for estimation by the accepted method.

TABLE II

Std. $MnSO_4$ soln. added, ml.	Equivalent Mn, g. × 10^{-5}	$KMnO_4$ soln., ml.			Equivalent Mn, g. × 10^{-5}	Error %
		Total	Blank	Nett		
2·5	0·77	1·25	0·55	0·70	0·72	− 6·5
"	"	1·25	0·55	0·70	0·72	− 6·5
3·0	0·92	2·40	1·45	0·95	0·97	+ 5·4
"	"	2·05	1·20	0·85	0·87	− 5·4
"	"	1·40	0·55	0·85	0·87	− 5·4
"	"	1·55	0·65	0·90	0·92	nil
"	"	1·55	0·65	0·90	0·92	nil
"	"	1·55	0·65	0·90	0·92	nil
"	"	1·55	0·65	0·90	0·92	nil
3·5	1·08	2·50	1·45	1·05	1·08	nil
"	"	2·50	1·50	1·00	1·02	− 5·55
"	"	2·25	1·20	1·05	1·08	nil
"	"	1·70	0·70	1·00	1·02	− 5·55
"	"	1·55	0·55	1·00	1·02	− 5·55
4·0	1·23	1·70	0·55	1·15	1·18	− 4·05
"	"	1·70	0·55	1·15	1·18	− 4·05
"	"	2·70	1·45	1·25	1·28	+ 4·05
"	"	2·70	1·50	1·20	1·23	nil
"	"	2·00	0·70	1·30	1·33	+ 8·1
5·0	1·54	2·95	1·45	1·50	1·54	< 1
"	"	3·00	1·50	1·50	1·54	< 1
"	"	2·65	1·20	1·45	1·48	− 3·9
"	"	2·25	0·70	1·55	1·59	+ 3·25
6·0	1·85	2·40	0·55	1·85	1·89	+ 2·2
"	"	2·40	0·55	1·85	1·89	+ 2·2
"	"	2·35	0·55	1·80	1·84	< 1
"	"	2·35	0·55	1·80	1·84	< 1
7·0	2·15	2·60	0·65	1·95	2·00	− 7·0
"	"	2·60	0·65	1·95	2·00	− 7·0
10·0	3·08	2·00	0·65	1·35	2·76	−10·4
"	"	2·05	0·65	1·40	2·87	− 6·8
"	"	2·10	0·70	1·40	2·87	− 6·8
"	"	1·95	0·60	1·35	2·76	−10·4
"	"	2·05	0·60	1·45	2·97	− 3·6
12·0	3·69	2·40	0·70	1·70	3·48	− 5·7
"	"	2·40	0·70	1·70	3·48	− 5·7
15·0	4·62	2·60	0·60	2·00	4·10	−11·2
"	"	2·80	0·60	2·20	4·50	− 2·4
"	"	2·90	0·70	2·20	4·50	− 2·2
"	"	2·70	0·70	2·00	4·10	−11·2

Note—In all the tests taking 10 ml. or more of standard manganous sulphate solution, the precipitated magnesium hydroxide, after settling, etc., was dissolved and diluted to 100 ml., of which 50 ml. were taken for the estimation.

Collection of manganese—That the method of collection by co-precipitation with magnesium hydroxide is satisfactory (within certain limits as laid down later) is borne out by the fact that manganese is not detectable in the liquor after one "extraction" with magnesium, and also by the good agreement between the quantities of manganese added to the soda samples and those found by the periodate method as described (see Table II).

Method of estimation—The periodate method as described was found satisfactory within the limits stated below. The colours developed were stable and remained so for at least 24 hours, there being no increase in depth of colour after half an hour from the time of oxidation.

Accuracy—Provided the permanganate solution (approximately 0·001 *N*) is standardised against standard manganese solution, the collection and estimation of the manganese is accurate to about ±7 per cent., within the limits of useful application of the method.

Limits—From Table II it appears that such an amount of caustic soda solution should be taken as will give a total manganese content of from $0{\cdot}8 \times 10^{-5}$ to $2{\cdot}0 \times 10^{-5}$ g.; less than $0{\cdot}8 \times 10^{-5}$ g. gives colours too pale, and more than $2{\cdot}0 \times 10^{-5}$ g. colours too deep for accurate matching.

For larger quantities of manganese the Spekker absorptiometer can be used with advantage, but it was found that with concentrations of manganese within the above limits the depth of colour produced under the conditions given above was too pale. It might be possible to use the instrument with a 20-cm. cell, but at the time of writing this model was not available.

Effect of chloride content—Sodium chloride solution was added to 6-ml. portions of the standard manganous sulphate solution to give chloride contents of 0·00001 and 0·0001 g. No detectable difference in tint was observed, and it was concluded that the chloride content of commercial caustic sodas would not interfere with the estimation of manganese.

SUMMARY—

A method is described for the estimation of very small quantities of manganese in commercial caustic soda, by collection on precipitated magnesium hydroxide and subsequent oxidation with periodate in phosphoric acid solution.

We wish to thank Messrs. Courtaulds Ltd. for permission to publish this paper, Mr. G. Hegan for advice and criticisms, and members of the Preston Laboratory Staff for help in testing the method on a routine basis.

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COURTAULDS LTD.
RED SCAR WORKS, PRESTON

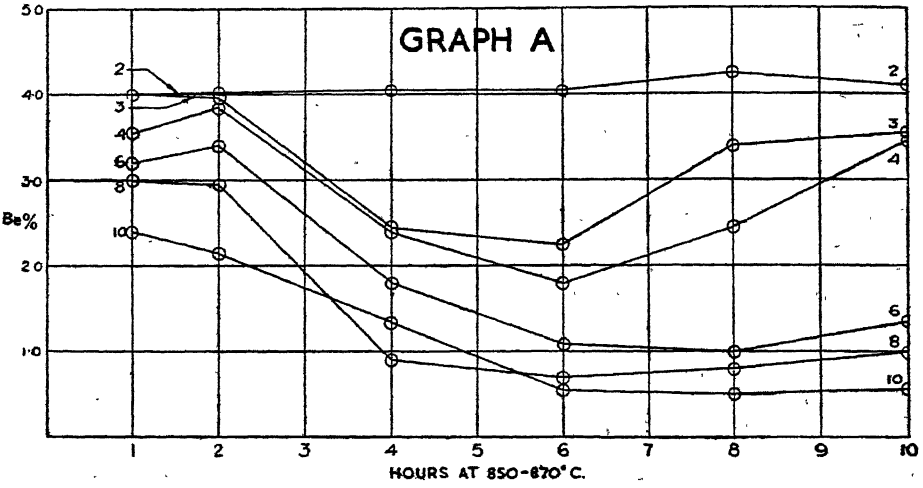
March, 1947

An Investigation into Factors affecting the Sodium Carbonate Fusion of Beryl

BY G. H. OSBORN

THE accurate determination of the beryllium content of beryl, $3BeO,Al_2O_3,6SiO_2$, has become of great commercial importance in recent years and considerable work, involving the use of *p*-nitrobenzene-azo-orcinol,[3] has been done on the subject by the author and Dr. W. Stross.[1,2] In these researches all the classical methods given for the attack on beryl, which is a very refractory material, were considered. It was found, surprisingly enough, that one of the most frequently recommended methods,[4,5,6,7] i.e., the sodium carbonate fusion, gave extractions so variable that attempts to use it were abandoned; a considerable amount of work had to be undertaken before a new and satisfactory method of attack by borax was found and published.[1]

Recently, however, the reason why such unsatisfactory extractions were obtained with the sodium carbonate fusion has been re-investigated by the author. On examining the literature it was found that whereas various ratios of sodium carbonate to beryl were given, most frequently 10:1, no details of temperature or time were mentioned, the direction frequently being to "fuse," without further amplification. It was therefore decided to carry out a full investigation of times, ratios and temperatures. An electric furnace (Catterson Smith) was used, fitted with two carefully calibrated pyrometers, the leads of one of which were at the base, next to the crucible, and the leads of the other at the top of the furnace chamber. The pyrometers differed very little in their readings and were a good check on each other. The ratios of sodium carbonate to 1 of beryl taken were 2, 3, 4, 6, 10,* and three ranges of temperatures were examined, A, 850° to 870° C.; B, 1000° C. ±10°; C, 1200° C. ±20°. After fusion the melts were, in all cases, extracted with perchloric or sulphuric acid, and the soluble beryllium contents determined by the *p*-nitrobenzene-azo-orcinol method.[1] The use of hydrochloric acid for extracting the melt was rejected owing to the very great risk of formation of the insoluble beryllium oxychloride, which would be removed with the silica and therefore lost. Hydrochloric acid has been recommended repeatedly for this purpose; the author carried out very many determinations with it, but did not once obtain a correct result, whereas with sulphuric or perchloric acid correct extractions were at all times obtained under suitable conditions. The beryl used was one that had been frequently assayed and found to have an average beryllium content of 4·3 per cent. It was ground to pass 150 mesh and intimately mixed with "AnalaR" sodium carbonate in platinum crucibles fitted with lids. All experiments in the same series were begun at the same time and the fusions were removed after the periods specified so as to have conditions as identical as possible.



The results obtained are shown in Graphs A, B and C, which relate to fusions at 850–870 °C., 1000° C. and 1200° C. respectively. The periods of fusion are shown along the abscissae, and the percentages of beryllium found along the ordinates. The number of each "curve" shows how many parts of sodium carbonate to 1 part of beryl were used in the fusions plotted on that particular "curve."

Experiment A (see Graph A)

It will be seen here that the ratio 2 : 1 gives an almost straight line throughout, whilst all the other ratios give very variable results. This is explained by the excess alkali producing the α-hydroxide of beryllium, which is gradually, but not completely, re-dissolved after prolonged heating. The lowest extraction was obtained at the most frequently recommended ratio 10 : 1.

Experiment B (see Graph B)

Here again the 2 : 1 ratio alone gives a correct result, whereas the other ratios give results which are extremely variable. For reasons of space, the graph shows results only up to 24 hours, but the 3 : 1 and 4 : 1 series were extended for 48 hours without achieving a better extraction than 3 per cent. of beryllium. The 4 : 1 series was done in duplicate; one of the duplicate series is represented by a broken "curve," and it will be seen that with excess of sodium carbonate present no reproducibility can be assured, even under identical conditions.

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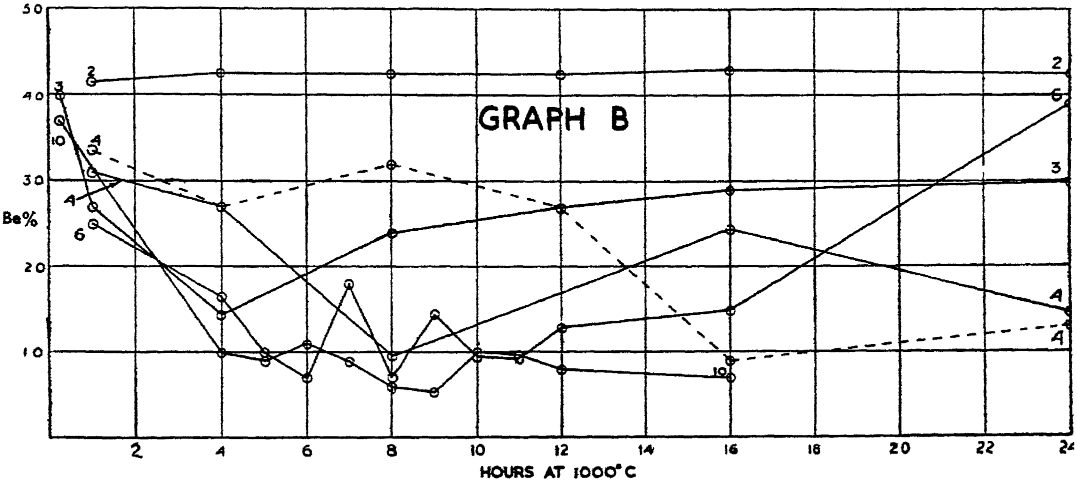
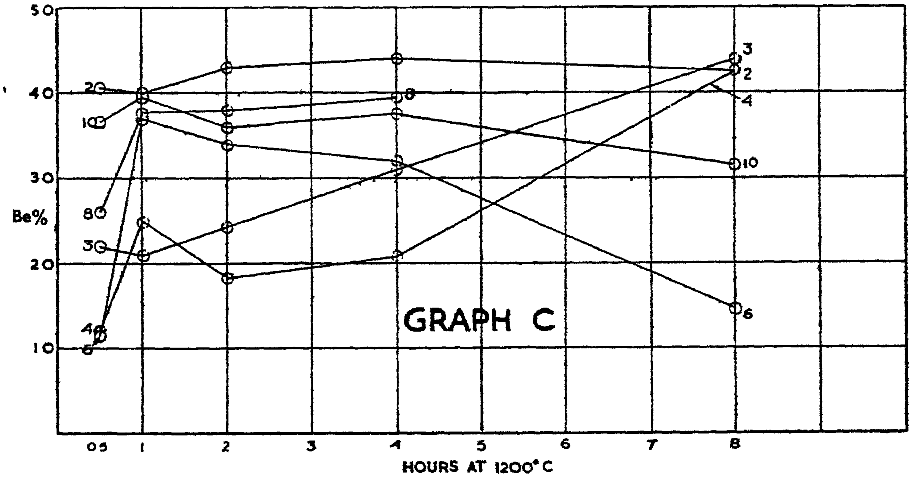
* The ratio 1 : 1 is not discussed here as it does not give complete extraction under any conditions, the quantity of alkali being insufficient.

Experiment C (see Graph C)

Here yet again the 2 : 1 ratio gives good results, and the other ratios give erratic results. The main difference from the previous experiments is that after 8 hours the effect of some of the ratios at this high temperature was to re-dissolve nearly all the α-hydroxide. This result could not, however, always be reproduced, whereas with the 2 : 1 ratio results were at all times reproducible.



Further examination of the literature showed that the insolubility of the α-hydroxide in sodium carbonate fusion was known, and attempts to utilise it were made,[8,9,10,11] but as these were based on total insolubility of the $Be(OH)_2$ it is not surprising that they were abandoned, for it may be clearly seen from the above graphs that even under the most carefully controlled conditions it is impossible to render all the beryllium insoluble. The real facts were, perhaps, better understood by the German workers, who based two patents[12,13] on the insolubility of the α-hydroxide in sodium carbonate. They attempted to use this reaction to separate beryllium from the aluminium and silica in beryl. The beryllium was to be recovered by boiling the α-hydroxide in concentrated sulphuric acid. However, the fact that these patents were never worked, and that the inventor subsequently adopted the lime extraction on a large scale in Germany, would seem to indicate that it was not as successful as was claimed, and an examination of the graphs above shows that the difficulty of obtaining even an 80 per cent. extraction under these conditions is very great.



SUMMARY—A study has been made of the factors affecting the sodium carbonate fusion of beryl, *i.e.*, the time and temperature of fusion and the ratios of sodium carbonate to beryl, and it has been shown that (*a*) the high ratios of sodium carbonate to beryl frequently advocated are to be avoided owing to the formation and precipitation of the α-hydroxide, which is insoluble in acid and, therefore, lost with the silica when this is removed by filtration, and (*b*) the only ratio that has been found to give reliable results over all ranges of temperature from 800° to 1200° C., and over all periods of heating is 2 parts of sodium carbonate to 1 part of beryl. A convenient time of fusion for normal experimental work is half an hour.

Thanks are due to Mr. G. Abrey who carried out much of the experimental work under the author's direction, to Mrs. G. Stross who made most of the photometric measurements, to Dr. W. Stross for many fruitful discussions and valuable suggestions and to the Directors of International Alloys Limited, in whose laboratories these experiments were carried out, for permission to publish. Thanks are also due to Mr. Noel L. Allport, F.R.I.C., of The British Drug Houses Ltd., London, for helpful discussion and advice with the preparation of the manuscript, and to the author's colleagues at The British Drug Houses Ltd., B.D.H. Laboratory Chemicals Group, Poole, for assistance with the preparation of the graphs.

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April, 1947

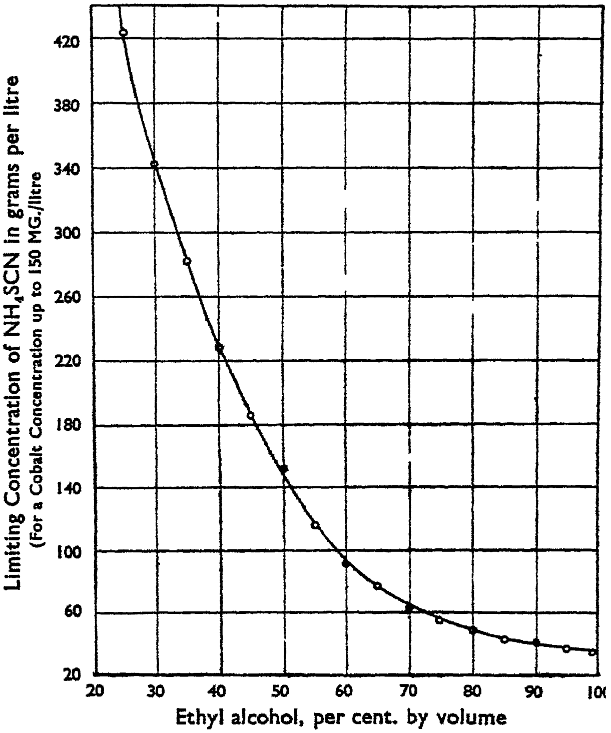
The Stability of the Cobaltous Thiocyanate Complex in Ethyl Alcohol-Water Mixtures and the Photometric Determination of Cobalt

BY NORBERT URI

TOMULA[1] was the first to propose the photometric determination of cobalt by means of its intensely blue thiocyanate complex. According to this method the cobalt is determined in a 50 per cent. (by volume) aqueous acetone solution containing 5 g. of ammonium thiocyanate per 100 ml., by comparing the extinction of this solution with that of a standard solution of similar cobalt concentration containing the same quantities of ammonium thiocyanate and acetone. As this method developed, special consideration was given to the disturbing effect of ferric ions: Kolthoff[2] proposes to remove them by transformation into the stable FeF_6''' complex; Ditz and Hellebrand[3] suggest their elimination by means of freshly precipitated calcium carbonate; Zemel,[4] for the analysis of sulphidic ores, proposes a mixture of sodium fluoride and sodium acetate; the use of pyrophosphate for the removal of iron and nickel is proposed by Lurie and Troitzkaja,[5] Lurie and Ginsburg,[6] and Zvenigorodskaya.[7] In all the investigations cited above, cobalt was determined according to Tomula's original procedure, in 50 per cent. aqueous acetone. Babicka[8] and Pevtsov,[9] on the other hand, suggest extraction of the blue $Co(SCN)_4''$ with a mixture of *iso*amyl alcohol and ethyl ether.

We investigated the stability of the cobaltous thiocyanate complex and worked out the conditions for the photometric determination of cobalt in mixtures of ethyl alcohol and water, and came to the conclusion that there was little if any advantage in the use of acetone over that of ethyl alcohol. The important point is that enough ammonium thiocyanate must be added, so that dissociation of the complex can be neglected. Under these conditions the colour intensity is the same when working with acetone or with ethyl alcohol.

Apparatus—The optical measurements were made with a Hellige Panphotometer. This apparatus is constructed on the principle of a visual abridged spectrophotometer with a movable light source. On one side, part of the light is absorbed by the sample solution (of constant layer depth), but on the other side—and in this the instrument differs from the Duboscq colorimeter—there is no standard solution. The intensity of the light reaching the two contiguous semi-circular fields of view of the ocular is equalised by moving the light source nearer to or farther from the sample solution by means of a hand-wheel. The hand-wheel is fitted with a scale, calibrated in values of $\log_{10}(I_0/I)$, allowing the direct reading of the extinction of the sample solution. The apparatus is provided with filters for the spectral regions of 690, 660, 570, 550, 530, 500, 470, and 450 mμ. The extinction can be measured in a solution layer of 1, 5, 10, 20, or 50 mm.



EXPERIMENTAL—We measured the extinction of 0·0025 *M* cobaltous nitrate solutions at various concentrations of ammonium thiocyanate and ethyl alcohol. The extinction, measured at a constant cobalt concentration, depends on the concentrations of both ammonium thiocyanate and ethyl alcohol, but if, at constant alcohol content, the concentration of the thiocyanate is increased, a certain limiting concentration is reached beyond which any further increase does not measurably influence the extinction of the solution. At this limiting concentration we assume the formation of the complex to be practically complete, so that dissociation may be neglected. It was found that at and above the limiting concentration, which varies with the alcohol concentration, the extinction, when measured in the spectral region of 570 mμ., *i.e.*, in the proximity of the extinction maximum, was exactly proportional to the concentration of cobalt for concentrations up to 0·0025 *M*, *i.e.*, 147·4 mg. of cobalt per litre. No accurate proportionality was obtained at higher cobalt concentrations. The value of $\log_{10}(I_0/I)$ for a 1-mm. layer of a solution containing 147·4 mg. of cobalt per litre, under the conditions of 100 per cent. thiocyanate complex formation, was found to be 0·280 in the spectral region of 570 mμ., corresponding to a molar extinction coefficient of 1120. Once the proportionality of the extinction with the concentration of the cobaltothiocyanate complex (up to ∿ 150 mg. of Co per litre) was established, it was easy to calculate, from extinction data at different concentrations of thiocyanate and ethyl alcohol, the degree of dissociation of the complex formed. (The colour of the cobaltous ion itself is altogether negligible at our working concentrations.) Thus we obtained a complete picture of the stability of the cobaltous thiocyanate complex in ethyl alcohol - water mixtures.

The results, in terms of percentage dissociation, are presented in Table I. Measurements were taken with an accuracy of ±1 per cent. For control purposes the extinction was measured immediately after the preparation of solutions and again after 24 hours, but the results obtained were the same. The graph in Fig. 1 shows the limiting concentration of ammonium thiocyanate in grams per litre over the entire range of ethyl alcohol - water mixtures. This graph should be taken into account in the analytical procedure. For concentrations of ethyl alcohol below 25 per cent. the limiting thiocyanate concentration exceeds the solubility of ammonium thiocyanate, and therefore accurate photometric determination is not practicable under these conditions. With alcohol concentrations above 25 per cent., however, the determination does give accurate results, provided the amount of thiocyanate added is not less than corresponds to the limiting concentration as shown in Fig. 1, and the cobalt concentration does not exceed about 150 mg. per litre. The extinction of the sample solution is compared with that of a standard solution of a cobalt salt which is determined gravimetrically. For the purpose of this investigation cobaltous nitrate solutions were determined electrolytically in presence of sodium citrate and acetic acid, according to a method developed by Spiegler[10] in these laboratories. It is not absolutely necessary that the cobalt concentration in the standard solution should be similar to that in the sample solution, provided it does not exceed 150 mg. per litre and the limiting concentration of ammonium thiocyanate is present.

TABLE I

DEGREE OF DISSOCIATION (IN PER CENT.) OF THE COBALTOTHIOCYANATE COMPLEX AT VARIE
CONCENTRATIONS OF NH_4SCN IN ETHYL ALCOHOL - WATER MIXTURES

Cobalt concentration 0·0025 *M* corresponding to 147·4 mg. Co per litre.

Molar concentration of NH_4SCN	Vol. per cent. ethyl alcohol										
	0	10	20	30	40	50	60	70	80	90	99
5·0	85·2	71·5	43·5	0							
4·0	89·6	78·7	55·4	10·5	0			Complete complex formation			
3·0	95·2	87·6	66·2	23·2	0						
2·0	100	95·5	83·0	55·8	12·7	0					
1·5		100	91·0	74·5	26·2	12·5	0				
1·0		100	96·5	86·9	55·7	33·4	3·6	0			
0·75			100	92·5	72·5	46·4	12·5	4·5	0	0	
0·50			100	96·5	84·2	66·2	29·8	14·3	8·9	3·6	0
0·20				100	100	92·0	66·2	52·5	33·3	13·2	7·1
0·10		Complete dissociation				100	90·2	64·3	45·0	24·3	16·5
0·05							100	90·0	75·2	58·3	33·0
0·02								100	97·5	88·3	70·5

Nickel forms a similar thiocyanate complex, which is (contrary to Tomula's assumptio no less stable than that of cobalt, as we proved by conductivity measurements; but i colour intensity is so weak in comparison with that of the cobaltous complex that we coul determine cobalt (using the yellow-green 570 mμ. filter) under our working conditions in i presence up to a molar ratio 1 Co : 2 Ni without loss of accuracy. The colour intensity the cobalt complex is so great that the metal can be determined at a concentration of onl 0·5 mg. per litre in a solution layer of 50 mm.

Procedure—Pipette 1 ml. of the aqueous cobalt sample solution (at a maximum co centration of 1·5 g. per litre) into a 10-ml. volumetric flask, add 3 ml. of an alcoholic ammoniu thiocyanate solution containing 150 g. per litre and dilute to the mark with 96 per cen ethyl alcohol. Determine the extinction of this solution and compare it with that of a standar cobalt salt solution, preferably in the spectral region of 570 mμ. If for any of various reason such as the solubility of other substances present, the ethyl alcohol concentration must reduced (working minimum 25 per cent.), ascertain the amount of ammonium thiocyanate which is to be added from the graph in Fig. 1.

SUMMARY—

The stability of the cobaltous thiocyanate complex has been determined at various concentrations of ethyl alcohol and ammonium thiocyanate. The degree of dissociation of the complex was calculated on the basis of photometric extinction measurements. Conditions were worked out for the photocolorimetric determination of cobalt by the thiocyanate method in ethyl alcohol - water mixtures. It was found that there were limiting concentrations of ammonium thiocyanate, depending upon the ethyl alcohol concentration, above which the cobalt is practically quantitatively converted into a complex compound. Under these conditions Beer's law is obeyed, and the photometric determination gives accurate results.

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Notes

AN INDICATOR CHANGING AT *p*H 0·5 FOR THE CONTROL OF SULPHIDE PRECIPITATION

VARIOUS suggestions have been made for securing a complete and clear-cut precipitation of the metals of the hydrogen sulphide group. Treadwell insisted that the solution should be about 0·3 to 0·4 *N* in respect of hydrochloric acid, and some such figure as this is generally accepted. This value is usually obtained either by keeping some check on the amount of acid previously used and adjusting the volume accordingly, or by neutralising and then re-acidifying, or by some rapid method of titration.[1]

But these methods do not take into account either the small acidity produced by the hydrolysis of salts of weak bases or the buffering effects of salts of any weak acids that may be present. This buffering effect may be considerable; acetates, for instance, can completely spoil the separation.

If the usual theory of sulphide precipitation is accepted, some rapid method of controlling the concentration of $H^{\cdot}$ ions at between *p*H 0·4 and 0·5 is required. Fowles[2] suggests and uses methyl violet as an indicator for this *p*H. Given an experienced operator with a good eye for colour, this indicator can be used to show the correct hydrogen ion concentration. A more satisfactory indicator may be made by dissolving 0·3 g. of haematoxylin and 0·06 g. of methyl violet 6B in 100 ml. of 20 per cent. *iso*propyl alcohol. This indicator is golden yellow at *p*H 0·5, green at *p*H 1 and orange-red at *p*H 0.

Without some standard of comparison or some experience of its use, it is not very easy to decide when this indicator shows exactly *p*H 0·5, but it is usually sufficient to know that if the solution shows any greenish tint more acid is required, and that any orange tint indicates excessive acidity.

One complication occurs when basic salts such as those of antimony and tin are present. The haematoxylin is adsorbed on the basic precipitate and the colour of the adsorbed dye changes to a deep mauve, although the acidity of the solution may correspond to the green tint of the mixed indicator. In presence of these basic salts methyl violet alone is a useful indicator, and gives a grass-green tint at *p*H 0·5. The solution may not be quite clear at this acidity, but sulphide precipitation will be complete.

Trials with these indicators have shown their usefulness. A mixture of stannous chloride and copper and manganese sulphates, with the metal ion concentration corresponding to

0·05 *M*, was acidified, with methyl violet as indicator, and precipitated with hydrogen sulphide in the usual way. The filtrate, after the usual preliminaries, was concentrated and divided. One part was tested for traces of tin (after reduction) by the cacotheline reaction, and the other for copper with ferrocyanide. No trace of these metals could be detected. The precipitated sulphides were washed and examined by fusion with sodium carbonate and nitrate, but no manganese could be found. The whole experiment was repeated but with addition of enough sodium acetate to make the mixture 0·5 *M* in respect of this salt. Again precipitation of the copper and tin was complete, and no manganese was brought down. In each experiment manganese was subsequently precipitated from part of the filtrate in the ammonium sulphide group, and came down with an unusually clean appearance.

An interesting feature was that when the mixed indicator was used it coloured the Group III solution, despite boiling off the hydrogen sulphide and boiling with a little dilute nitric acid. Another solution was prepared, without the tin because of the haematoxylin complication, but with added aluminium. After adjustment of the acidity with the mixed indicator present, separation of the sulphides, and boiling and oxidising in the usual way, the aluminium came down in the ammonium hydroxide group characteristically coloured by the haematoxylin and leaving the solution colourless. The coloration of the precipitate greatly facilitated its detection when only a little was present.

In the separation of zinc from cadmium with use of this indicator it was found that, after precipitation from 0·05 *M* solutions of the metals and filtration from the sulphide precipitate, the filtrate was quite free from cadmium, so far as could be ascertained by concentration and spot-testing with diphenylcarbazide reagent, but the cadmium sulphide retained some zinc, which could not be removed by washing twelve times with boiling distilled water. When the zinc concentration was reduced to 0·01 *M*, cadmium sulphide still brought down a little zinc with it—enough to be detected with the mercuric ammonium cobalt thiocyanate reagent.* This is not necessarily a disadvantage, because an adequate quantity of zinc remains to be detected in the ammonium sulphide group. The explanation of the co-precipitation with cadmium is uncertain, but the possibility of the formation of a mixed salt as an alternative to adsorption must not be overlooked.

These experiments suggest that the use of a mixed indicator showing clearly when the *p*H of a solution is close to 0·5 is advantageous in improving the accurate separation of the Group II metals and also makes aluminium easier to detect. Certainly the indicator saves time.

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June, 1947

DETERMINATION OF CALCIUM OXIDE IN LOW-LIME REFRACTORY MAGNESIA

THE oxalate - permanganate method outlined in a previous note[1] for the determination of lime in refractory dead-burned magnesia gives low results when less than 3 per cent. of lime is present. Although certain analysts[2] claim that amounts of calcium of this order cannot be satisfactorily determined by direct precipitation as oxalate, subsequent work has shown that the method can be modified to give accurate results down to a lime content of only 1 per cent.

Solutions corresponding to those obtained by dissolving magnesias containing 1 to 3 per cent. of lime in hydrochloric acid were prepared, and calcium was determined by carrying out two precipitations of calcium oxalate without removing iron and aluminium. Each solution (300 ml.) was heated, 50 g. of ammonium oxalate were added and dissolved with stirring, and after addition of methyl red solution calcium oxalate was precipitated at about 90 °C. by adding concentrated ammonia dropwise until the colour changed to yellow. The solution was maintained at about 90° C. for 60 minutes and then filtered hot. After washing with cold 0·1 per cent. ammonium oxalate solution the precipitate was dissolved in 20 ml. of hot dilute hydrochloric acid containing 5 ml. of the concentrated acid, 1 g. of ammonium oxalate was added and calcium oxalate reprecipitated in a volume of 250 ml. by dropwise addition of concentrated ammonia at the boiling point until the methyl red became yellow. The solution was gently boiled for 30 minutes and then set aside at room temperature for 1½ hours. The precipitate was washed with water and subsequently titrated with 0·1 *N* potassium permanganate. The results obtained are given in Table I.

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* To the solution to be examined add an equal volume of a 0·02 per cent. aqueous solution of cobalt chloride followed by the same volume of an aqueous solution containing 2·7 per cent. of mercuric chloride and 3·0 per cent. of ammonium thiocyanate; the presence of zinc is indicated by formation of a blue crystalline precipitate.[3]

TABLE I

Composition of solutions	CaO present g.	CaO found g.
0·995 g. MgO as $MgCl_2$ 0·020 g. Fe_2O_3 as $FeCl_3$ 0·022 g. Al_2O_3 as $AlCl_3$ 0 to 0·0329 g. CaO as $CaCl_2$ 0·02 g. SiO_2 5 ml. hydrochloric acid Water to 300 ml.	0, 0	0·0007, 0·0007
	0·0055, 0·0055	0·0034, 0·0034
	0·0055, 0·0055	0·0054, 0·0054
	0·0110, 0·0110	0·0109, 0·0111
	0·0110, 0·0110	0·0109, 0·0109
	0·0219, 0·0219	0·0225, 0·0225
	0·0219, 0·0219	0·0223, 0·0223
	0·0329, 0·0329	0·0326, 0·0333
	0·0329, 0·0329	0·0330, 0·0329

When the lime present amounted to 0·0110 g. or more there was thus complete recovery. Similar results were obtained when the amount of ammonium oxalate used in the first precipitation was reduced to 25 g., but increasing the amount of hydrochloric acid used in this precipitation tended to produce low and varying figures.

Solutions were also prepared, corresponding to those obtained by alkali fusion of magnesias containing 1 to 3 per cent. of lime and subsequent solution of the melts in hydrochloric acid, and calcium was determined in them as above. Low figures were obtained with solutions containing less than about 0·02 g. of CaO, but it was found that correct results could be obtained with those containing only 0·01 g. of CaO, corresponding to a magnesia containing 1 per cent. of lime, if the first precipitation was made at the boiling point and the solution then gently boiled for 2 hours. Results obtained with this modification are given in Table II.

TABLE II

Composition of solutions	CaO present g.	CaO found (by titration) g.	CaO found (by weighing) g.
0·961 g. MgO as $MgCl_2$ 0·020 g Fe_2O_3 as $FeCl_3$ 0·021 g. Al_2O_3 as $AlCl_3$ 0 to 0·0323 g. CaO as $CaCl_2$ 0·02 g. SiO_2 5 ml. hydrochloric acid 6 g. NaCl (≡5 g. Na_2CO_3) Water to 300 ml.	0, 0	0·0006, 0·0006	
	0, 0		0, 0
	0·0054, 0·0054	0·0043, 0·0045	
	0·0053, 0·0053		0·0034, 0·0044
	0·0108, 0·0108	0·0107, 0·0109	
	0·0106, 0·0106		0·0102, 0·0110
	0·0215, 0·0215	0·0219, 0·0221	
25 g. $(NH_4)_2C_2O_4.H_2O$ used in first precipitation	0·0213, 0·0213		0·0212, 0·0214
	0·0323, 0·0323	0·0329, 0·0331	
	0·0319, 0·0319		0·0320, 0·0320

Figures obtained by igniting the precipitate to calcium oxide agreed closely with those obtained by titration with permanganate.

On the basis of the above results the following procedure is recommended for the determination of lime in refractory magnesias containing from 1 to 3 per cent. of CaO.

PROCEDURE—

Grind the sample to pass a 200-mesh B.S.I. sieve, and dissolve 1 g. in hot dilute hydrochloric acid containing 10 ml. of the concentrated acid. Boil with a few drops of nitric acid to oxidise ferrous iron.

Dilute to 300 ml., heat, add 25 g. of ammonium oxalate and dissolve by stirring. Add methyl red solution, and precipitate calcium oxalate at about 90° C. by dropwise addition of concentrated ammonia until the colour changes to yellow. Maintain at about 90° C. for 60 minutes. Filter hot, and wash thoroughly with cold 0·1 per cent. ammonium oxalate solution. Dissolve the precipitate in 20 ml. of hot dilute hydrochloric acid, containing 5 ml. of the concentrated acid, and wash the filter paper with hot diluted hydrochloric acid (1 in 100).

Add 1 g. of ammonium oxalate, dilute to 250 ml., heat to boiling and precipitate calcium oxalate as above. Boil gently for 30 minutes, and then set aside at room temperature for 1½ hours. Filter, wash thoroughly with cold water, and titrate with 0·1 *N* potassium permanganate solution.

If the sample will not dissolve in hydrochloric acid, fuse 1 g. with 5 g. of anhydrous sodium carbonate and dissolve the cold melt in dilute hydrochloric acid containing 20 ml. of the concentrated acid. Boil with a few drops of nitric acid to oxidise ferrous iron.

Dilute to 300 ml., add 25 g. of ammonium oxalate and heat to the boiling point. Add methyl red solution and precipitate calcium oxalate by dropwise addition of concentrated ammonia until the colour changes to yellow. Boil gently for 2 hours, allow to cool slightly, and filter hot. Wash thoroughly with cold 0·1 per cent. ammonium oxalate solution, dissolve the precipitate in acid, carry out a second precipitation, and complete the determination as described above.

This procedure was tested on samples of two commercial refractory magnesias with the following results:—

Sample	CaO per cent. found by Solution in HCl	CaO per cent. found by Alkali fusion	CaO per cent. found by another method
Dead-burned sea-water magnesia ..	1·97	1·95	1·95
Calcined Indian magnesite	1·08	1·11	1·03

Solution of the samples in hydrochloric acid gave almost the same results as fusion with sodium carbonate followed by solution in hydrochloric acid; this was to be expected, since both materials were found to be completely decomposed by hydrochloric acid. The actual lime content of each was determined by dissolving in acid, removing ferric oxide and alumina by two precipitations with ammonia, increasing the amount of lime present by adding standard calcium chloride solution, carrying out two precipitations of calcium oxalate and subsequently titrating with permanganate. It is well known that when the ratio of calcium to magnesium is not very small the oxalate separation is satisfactory, and the actual lime content was therefore obtained by subtracting the amount added as calcium chloride from the amount determined by titration. As can be seen, the actual amounts of lime present closely corresponded with those obtained by the method given under "Procedure."

I wish to thank the Research Manager and the Directors of the British Periclase Company for permission to publish this note.

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PALLISER WORKS
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ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Methods of Fat Determination in Dairy Products. L. C. Janse (*Chem. Weekblad*, 1946, **42**, 284–288)—A critical comparison of the three chief methods for fat determination has been made. *The Röse - Gottlieb method*—Results obtained by the Röse - Gottlieb method tend to be high, partly through oxidation of the fat by peroxides in the ether, and partly through extraction of non-fatty substances. *The Weibull method*—The official method of the Committee of the Dutch Dairy Association (*Off. Org. van Feder. Nederl. Zuivelfab.*, 1941, 569–570, 581–583, 594–596), modified in a few details by the author, is as follows. Transfer 25 g. of milk to a beaker, add a little pumice, and 50 ml. of 4·8 *N* hydrochloric acid. Cover with a clock glass, and boil gently for 20 min. Rinse down the clock glass with hot water, and pass the contents of the beaker through a wet and well-fitted, fat-free filter paper (11 cm. diameter, free from holes). After washing free from acid, dry the filter paper at 100° C. for 1 hr., a watch glass being placed under the tip of the filter to catch any molten fat running through. Transfer the filter paper to a glass extraction tube having a number of small holes in the bottom, support the paper on fat-free cotton-wool, and cover it with a layer of the wool. Rinse the funnel and watch glass with light petroleum (b.p. 40° to 60° C.), and extract the filter paper with the light petroleum in a continuous extraction apparatus for 4 hr. into a dried weighed flask. Dry the fat for 2 hr. at 102° to 105° C. and to constant weight (less than 2 mg. loss on 0·5 hr. further heating). Application of the method to other dairy products requires modifications in the preliminary acid treatment. For buttermilk, take double quantities of sample and 4·8 *N* acid. For cream, take 3 to 5 g. of sample, 40 ml. of water and 30 ml. of 25 per cent. hydrochloric acid. For cheese, take 3 g. of sample, 25 ml. of water and 30 ml. of 25 per cent. hydrochloric acid and boil for 1 hr. For full-cream milk powder take 2·5 g. of sample, 20 ml. of water and 30 ml. of 25 per cent. acid, and for skimmed-milk powder double quantities of each. For condensed milk, dilute to the concentration of fresh milk and treat like this. *The Gerber method*—The Committee concluded that two errors led to high results by this method. The first, due to neglecting the volume of the meniscus, is small. The second error runs parallel with the fat content of the milk, amounting to 1·9 per cent. of the amount found. It may be compensated by allowing a correction either by taking 10·79 ml. of milk in place of 11 ml., or by multiplying the result by 0·981. This correction factor is a mean value, and agrees with the finding of an investigation by the author. G. M.

Puerto Rican Fatty Oils. VI. Characteristics and Composition of "Molinillo" Seed Oil. C. F. Asenjo, J. A. Goyco, and Z. Martinez-Pico (*J. Amer. Chem. Soc.*, 1945, **67**, 1936–1937)—The "molinillo," known also as lion's ears, "quina de pasto" and "botón de cadeta," is a mint plant (*Leonotis nepetæfolia* L.) growing abundantly as a weed in Puerto Rico. Its oil had the following characteristics: sp.gr. 25/25, 0·8984; n^{20}, 1·4673; iodine value (Hanus), 82·5; saponification value, 191·2; acid value, 11·20; Reichert - Meissl value, 0·29; Polenske value, 0·15; unsaponifiable matter, 3·09 per cent.; acetyl value, 4·87. The percentages of fatty acids and their glycerides, respectively, were: linoleic, 11·9, 12·4; oleic, 64·6, 67·6; myristic, 1·3, 1·4; palmitic, 12·0, 12·6; stearic, 1·2, 1·3. E. B. D.

Limit Tests for Impurities. F. Reimers and K. R. Gottlieb (*Contributions from the Danish Pharmacopoeia Commission. Vol. I. Ejnar Munksgaard, Copenhagen*, 1946)—It is not possible to abstract all the information contained in this publication (*cf.* review, p. 500), but some of the more interesting points may be noted. *Chloride*—Rapid mixing is essential to obtain a reproducible turbidity, and the solution under test should be poured all at once into the 0·1 *N* silver nitrate solution. Salts have little effect on the sensitivity, but nitric acid should be present in salt-free solutions. *Sulphate*—To obtain reproducibility, the reaction mixture must be "seeded" with barium sulphate. One drop of sulphate solution (100 µg. per ml.) is placed at the bottom of a test tube, and 1 ml. of 0·5 *N* barium chloride solution is added, with shaking. After 1 min., 10 ml. of the solution under test are added and the mixture is shaken violently for 10 sec. The comparison is made after 5 min. *Calcium*—"Seeding" with calcium oxalate is also to be recommended here. *Heavy metals*—Various factors affect the colours in a different way for different metals. The dithizone reagent is less suitable than sodium sulphide for a limit test, partly because the sensitivity is too high, but also because the colours of the complexes with different metals vary greatly. *Ammonia*—The solution under test, mixed with an equal volume of sodium hydroxide solution, is placed in a test tube. In the mouth of the tube is suspended, on a glass hook, a piece of red litmus paper. After heating in a water-bath for 5 min., the colour of the litmus paper is compared with that of a test paper treated similarly with standard ammonia solution. *Carbonates*—This test is similar to that for ammonia. The carbonate is decomposed by acid, and the test paper used is filter paper moistened with a mixture of 0·05 *N* sodium hydroxide and phenolphthalein.

Colour standards—Cobalt, iron, and copper solutions similar to those of the United States Pharmacopoeia are used, but all dilutions are made with acid so that the hue is not changed by dilution. *Limits*—In addition to the ordinary limit test ("Limit A"), if it is prescribed that a substance must not show any reaction for an impurity then the comparative control known as "Limit B" must be used. An example of the thoroughness of the tests is shown by the proposed monograph for distilled water: From 500 ml. of distilled water, 100 ml. of water are distilled off in a glass apparatus connected by means of ground-glass joints. These 100 ml. are denoted as P_2. Subsequently, another 300 ml. are distilled. The non-distilled residue is denoted as P_1. In the tests for colour, sulphate, calcium, and metals, the tests are performed on P_1, whilst P_2 is used for the controls. In addition, there are tests for carbon dioxide, reaction to methyl red, reducing substances, chloride, nitrate, sodium, ammonia, and non-volatile matter. G. M.

Assay for Choline Chloride in Pharmaceutical Products. W. C. Gakenheimer and R. M. Regnera (*J. Amer. Pharm. Assoc., Sci. Ed.*, 1946, **35**, 311–312)—The recent interest in the oral administration of choline for the treatment of certain diseases of the liver (Brown and Muether, *J. Amer. Med. Assoc.*, 1942, **118**, 1403; Russakoff and Blumberg, *Ann. Internat. Med.*, 1944, **21**, 848; Richardson and Suffern, *Brit. Med. J.*, 1945, i, 156) has indicated the need for an accurate assay of choline chloride in pharmaceuticals. The use of phosphotungstic acid as a precipitant has been found to be rapid and trustworthy.

Procedure—(*a*) *For liquid samples* (*solutions in water, elixirs, syrups, etc.*)—Transfer such a volume of the sample as might be expected to contain about 1 g. of choline chloride to a 100-ml. volumetric flask and heat on a steam-bath at reduced pressure (100 to 125 mm. of mercury) to drive off moisture and any interfering decomposition products such as ethylamine, if present. While it is still warm, shake the residue with 50 ml. of absolute alcohol, and dilute to 100 ml. with the same solvent. Mix thoroughly, allow to settle, transfer 5 ml. of the clear, supernatant fluid to a beaker containing 10 ml. of a filtered, 10 per cent. solution of phosphotungstic acid in absolute alcohol, digest the mixture on a steam-bath for 5 min. and filter through a fine-porosity, sintered-glass crucible. Wash the residue on the filter with 5 ml. of absolute alcohol, dry at 55° C. for 2 hr., and weigh.

Weight of precipitate × 0·1375 = weight of choline chloride.

(*b*) *For solid samples* (*tablets, capsules, etc.*)—Extract an accurately weighed portion, expected to contain about 0·5 g. of choline chloride, in a micro-Soxhlet apparatus with 20 ml. of absolute alcohol for 3 hr. Transfer the extract quantitatively to a 50-ml. volumetric flask, distil off the alcohol, and heat the residue at 100° C. under reduced pressure (100 to 125 mm. of mercury) for 15 min. Dissolve in absolute alcohol, dilute to 50 ml., and continue the assay as described above.

The factor, 0·1375, for converting the weight of choline phosphotungstate to choline chloride is higher than the theoretical figure of 0·1291 to compensate for the slight solubility of the phosphotungstate in absolute alcohol under the assay conditions described. Different batches of phosphotungstic acid have been examined under the same conditions; the weights of precipitate obtained varied by only ±0·7 per cent. It is stated that although pure, anhydrous alcohol was used in the experiments, anhydrous, denatured alcohol is satisfactory. It is advisable to conduct a blank determination to establish the absence of interfering substances in the preparation under examination; no such source of error has been encountered in several common vehicles tested. A satisfactory recovery of choline chloride is obtained. J. A.

Biochemical

Step-Photometric Determination of Histidine. K. Schmid (*Helv. Chim. Acta*, 1946, **29**, 226–228)—The method, which can be used to determine histidine in impure solutions, such as urine, is a modification of that of Kapeller - Adler (*Biochem. Z.*, 1933, **264**, 131) in which a slight excess of bromine is added to the histidine-containing solution and the resulting dye is developed by means of an ammonia - ammonium carbonate mixture, and determined photometrically. In the present method, the excess of bromine is removed by addition of arsenious acid (Conrad and Berg, *J. Biol. Chem.*, 1937, **117**, 350), the urine is strongly diluted before test, and the bromination is carried out in presence of an excess of acid.

Procedure—Dilute 24-hr. rat urine to 100 ml. with water. Treat 1-ml. portions of this diluted urine with increasing volumes of a histidine solution containing 100 mg. of histidine and 2 ml. of 2 *N* sulphuric acid per 100 ml. Also, prepare 0·04 *N* sulphuric acid and use this to dilute to 2 ml. urine samples containing histidine. Add dropwise a solution containing 2·5 ml. of bromine, 250 ml. of glacial acetic acid, and 750 ml. of water, until the slight yellow colour of the liquid after 10 min. standing shows that there is a slight excess of bromine present. After 10 min., add 2 drops of 10 per cent. aqueous ammonia solution saturated with arsenious acid and 2 ml. of an ammonia - ammonium carbonate solution (2 parts of concentrated ammonia + 1 part of 19 per cent. ammonium carbonate solution). Heat in boiling water for 5 min., cool, and allow to stand at room temperature for 10 min. to develop the colour. Dilute to 10 ml. with the ammonia - ammonium carbonate solution, and determine the intensity of the colour with a step-photometer, using an S50 filter and a 5-mm. cell. As the compensating liquid use urine treated in the same way. For 0·4 to 1·6 mg. of histidine hydrochloride per millilitre in the diluted urine there is a linear relationship between the histidine content and the extinction.

The method is ten times less sensitive than that of Edlbacher *et al.* (*Z. physiol. Chem.*, 1941, **270**, 158), but has the advantage of being specific for histidine. E. M. P.

Method for the Estimation of Barbituric and Thiobarbituric Acids in Biological Materials. J. Raventos (*Brit. J. Pharmacol.*, 1946, **1**, 210–214)—In order to study the fate and distribution of barbiturates in the animal body, several methods of estimation were examined, but none was completely satisfactory. Using the methods of Levvy (*Biochem. J.*, 1940, **34**, 73), Delmonico (*Proc. Mayo Clin.*, 1939, **14**, 113), and Anderson and Essex (*Anaesthesiology*, 1943, **4**, 113) the recoveries of known amounts of barbiturates added to blood or tissues were low. A method was developed for the estimation of barbituric acid based upon Koppanyi's colour reaction (*J. Amer. Pharm. Assoc.*, 1934, **22**, 1076), and for the estimation of thiobarbituric acids on a colour reaction described by Cowan.

Procedure—A. *Extraction*—Using blood, take 10 to 20-ml. volumes of oxalated blood and mix with equal volumes of water and of a 10 per cent. solution of sodium dihydrogen phosphate. Extract with ether in a continuous extractor at 45° to 50° C. for 8 to 10 hr. Evaporate the ether extract to dryness. For urine, acidify to *p*H 5 with concentrated hydrochloric acid and extract with ether in a similar manner. When using tissues, take samples of 10 to 20 g., grind in a mortar with sand, and then mix with 10 per cent. of solid sodium dihydrogen phosphate. Allow to stand for 5 to 10 min., add 20 g. of anhydrous sodium sulphate for every 10 g. of tissue with continuous grinding. Transfer to a desiccator containing calcium chloride for one hr. Extract the dry powder for 2 to 3 hr. with 50 ml. of benzene in a well-stoppered flask. Filter and wash three times with 10 to 15 ml. of benzene. Concentrate the filtrate and washings to about 5 ml. by distillation at 50° C. under reduced pressure.

B. *The separation of barbituric and thiobarbituric acids*—The extracts must first be purified by chromatography. Dissolve the residues obtained from the extraction of urine or blood in 5 ml. of chloroform and dry with 1 to 2 g. of anhydrous sodium sulphate. Add the solutions to columns of activated alumina ⅜ in. × 4 in. The activated alumina used was grade O supplied by Messrs. Peter Spence of Manchester, and was treated by boiling 1200 g. with 1800 ml. of 10 per cent. acetic acid for 2 hr., filtering and washing with at least 20 litres of hot, distilled water. The alumina was dried, heated until the temperature reached 360° C., and then partly de-activated by adding 2½ per cent. w/v of water. Filter the chloroform solutions on to the columns and wash the flask and filter 3 times with 5 ml. of chloroform, pouring the washings on to the column. Wash the column with chloroform until the eluate is free from pigment. When tissues are used, pass the benzene extract through alumina columns with slight suction, washing the flasks with benzene and using benzene to elute the pigment from the columns, and finishing with 20 ml. of chloroform. The chloroform and benzene washings are discarded. Thiobarbituric acids are recovered from the columns by elution with 50 ml. of 2 per cent. methanol in chloroform, the eluates being kept for estimation. To elute the barbituric acids, use 50 ml. of 10 per cent. methanol in chloroform. Under these conditions separation is complete, and the recoveries of both acids are almost theoretical.

C. *Estimation*—Evaporate the eluates to dryness under reduced pressure at 40° to 50° C. and dissolve the residues in chloroform.

Thiobarbituric acids—Take an aliquot of the final chloroform solution in a test tube, and for every 2 ml. add 0·2 ml. of a 10 per cent. solution of diethylamine in methanol followed by 0·5 ml. of a saturated solution of anhydrous copper sulphate in methanol. A green colour develops at once and is stable for about 2 hr. Compare the samples in a suitable colorimeter with a series of similarly treated standard solutions of the thiobarbituric acid to be estimated, containing 0·03 to 0·5 mg. per ml. Tissues such as brain and liver give a blank value sometimes as high as 1 mg. per 100 g. of tissue. The reaction is fairly selective, not being given by malonic acid, theophylline, theobromine, thiourea, caffeine, guanine, uric acid, urea, creatinine, oxamide, succinic acid, lecithin, cholesterol, cystine, or glutathione.

Barbituric acids—For every 2 ml. of the final chloroform solution add 0·6 ml. of 5 per cent. *iso*propylamine in methanol followed by 0·1 ml. of a 1 per cent. solution of cobalt acetate in methanol. A reddish colour is produced and can be compared in a colorimeter with a series of similarly treated standards containing 0·1 to 1 mg. of the appropriate barbituric acid per ml. of chloroform. The recovery of known amounts of barbituric or thiobarbituric acids added to blood and tissues is approximately complete except when the amount from a 10-ml. sample is less than 0·3 mg.; recovery may then fall below 95 per cent. R. H. T.

Chemical Assay of Penicillin. M. Mundell, H. Fischbach, and T. E. Eble (*J. Amer. Pharm. Assoc., Sci. Ed.*, 1946, **35**, 373–378)—Methods for the assay of penicillin are reviewed, and the occurrence of the four types of penicillins G, K, F, and X, is pointed out. Data are given of the relative activities of these substances against *Staphylococcus aureus in vitro*. The following chemical methods were investigated:—the colorimetric method of Scudi (*J. Biol. Chem.*, 1946, **164**, 183), the penicillinase method (Murtaugh and Levy, *J. Amer. Chem. Soc.*, 1945, **67**, 1042), the methods proposed by the Chas. Pfizer Co. based upon alkali inactivation and hydrogen peroxide inactivation, a spectrophotometric method (Herriott, *J. Biol. Chem.*, 1946, **164**, 725), and the iodimetric method proposed by the Squibb Institute for Medical Research (Alicino, *Ind. Eng. Chem.*, *Anal. Ed.*, 1946, **18**, 619; *Abst.*, Analyst, 1947, **72**, 68).

It was found that the penicillinase method and the iodimetric method gave the most consistent results when applied to diverse samples of commercial penicillin. The paper describes both methods in full, but points out that the iodimetric method gives better results generally and is technically simpler and more straightforward. The authors examined the factors involved in the iodimetric reaction of the alkaline products of inactivation of sodium penicillin G and found that 2·52 to 2·53 ml. of 0·01 *N* iodine are consumed by the inactivation product from 1 mg. of crystalline sodium penicillin G. The following method is proposed for the assay of ampoules of penicillin.

Procedure.—Place 5-ml. aliquots of a solution of penicillin (approximately 1000 units/ml.) in each of two iodine flasks. To one, add an equal volume of *N* sodium hydroxide and allow to stand at room temperature for 15 min. and then add 5 ml. of 1·1 *N* hydrochloric acid and 15 ml. of 0·01 *N* iodine. After 15 min., titrate the excess of iodine with 0·01 *N* sodium thiosulphate, approaching the end-point with the use of starch indicator and the addition of 5 ml. of carbon tetrachloride. To the second flask add 15 ml. of the 0·01 *N* iodine and titrate immediately to serve as a blank determination. The difference between the two titration values divided by 2·52 gives the number of milligrams of penicillin present. The use of the 1·1 *N* solution of hydrochloric acid results in a medium of low *p*H which has been shown to be advantageous for iodimetric titrations. No adjustment of *p*H is made to the blank determination since this would result in the formation of degradation products that would react with iodine and yield a correspondly low value for the penicillin potency.

A series of batches of sodium penicillin G was assayed by this procedure and recoveries ranging from 99 to 101·6 per cent. of the theoretical were obtained. A few samples of penicillins F, X, and K were assayed both by the iodimetric and penicillinase methods, and the results indicate that a similar number of equivalents of iodine react with equimolecular quantities of the respective penicilliates resulting from alkaline inactivation. The work of the Squibb group suggested that penicillin X might give abnormally high values, but the results obtained did not show this. Comparative data obtained for samples of crystalline penicillin G using the iodimetric, penicillinase, and bio-assay methods are given. The results show good agreements between the chemical methods, the average recoveries being 99·8 per cent. for the penicillinase, 99·2 per cent. for the iodimetric, and 98·2 per cent. for the bio-assay method. The standard deviations from the known weight of penicillin were, respectively, 1·5 per cent., 1·2 per cent., and 4·8 per cent. Good agreement was similarly obtained with samples of commercial penicillin.

The results of the chemical methods are most accurately expressed in milligram-equivalent weights of penicillin without any reference to the type of penicillin involved. Since the molecular weights of the various known penicillins are of the same order, little error is to be anticipated by converting the results to milligrams by multiplying by the average of the molecular weights of the four penicillins. The results cannot be accurately translated to the unit basis without knowing the proportions of the four penicillins present. Hence, comparisons of the accuracy of the chemical and biological methods must be made upon material of known composition. The penicillinase method requires the use of special equipment, and is difficult to apply to some preparations of penicillin. It is probably safe to assume that it is highly specific, and methods based upon its action are specific for penicillin. The iodimetric method requires no special apparatus or reagents, and can be successfully applied to most preparations of penicillin, but is not specific for penicillin, so that in the absence of information on the composition of the sample, supplementary analysis might be required.

R. H. T.

Three-Hour "Physical Development" Cup-Plate Assay for Penicillin. F. M. Goyan, J. Dufrenoy, L. A. Strait, R. Pratt, and J. Juntunen (*J. Amer. Pharm. Assoc., Sci. Ed.*, 1947, **36**, 65–68)—A practical method for the assay of penicillin depending on the fact that cells of *Staphylococcus aureus* differ in their affinity for silver, supplied in the form of an aqueous solution of silver nitrate, according to whether they are under the influence of penicillin or not, is described. It has been found that the silver-impregnated plates are analogous to photographic emulsions, and the application of principles of photographic development produces images that show a marked difference between zones of inhibition and non-inhibition with a clearly defined boundary between the zones after only 5 hr. incubation. If the seeded plates are incubated for 3 hr. before the beginning of the assay, satisfactory images are produced after only 3 hr. incubation, and normal results have been obtained using such "pre-incubated" plates after storage for 5 days in a refrigerator.

Procedure—Prepare and seed the plates as for the standard cup-plate method of assay of penicillin, using *Staph. aureus*, NRRL Strain No. 313 (F.D.A. Strain No. 209P), incubate at 38° C., and store in a refrigerator until needed. Transfer the solutions to be tested to cylinders on the plates in the usual manner and re-incubate at 38° C. for 3 hr. After removal from the incubator, flood each plate with about 30 ml. of a nearly neutral, 0·1 per cent. aqueous solution of silver nitrate, expose for 2·5 to 3 min. to the illumination from two 40-watt daylight fluorescent lamps mounted in a reflector at a distance of about 35 cm. (this is equivalent to about 350 foot-candles). Remove the excess of silver nitrate solution by means of a suction device and then add 30 ml. of physical developer prepared as described below. After allowing the developer to act for 7 to 10 min., measure the diameters of the zones of inhibition in the conventional way.

Preparation of Physical Developer. Stock Solution A—Dissolve 80 g. of sodium thiosulphate and 20 g. of anhydrous sodium sulphite in 300 ml. of distilled water and add to this solution, slowly with constant stirring, a solution of 8 g. of silver nitrate in 200 ml. of distilled water. *Stock Solution B*—Dissolve 20 g. of sodium sulphite in 500 ml. of distilled water and, when dissolution is complete, add 3·8 g. of 2 : 4-diaminophenol hydrochloride and stir until dissolved. Store both stock solutions in a refrigerator and, just before use, mix equal volumes of solutions *A* and *B* and dilute their combined volume with 3 volumes of distilled water. The dilution of the developer and the time of development should be determined empirically for each laboratory, to allow for varying conditions of light intensity, temperature of development, etc. The concentration of silver nitrate used for impregnating the plates must be not less than 0·1 per cent. nor greater than 0·13 per cent., whilst the time of exposure must be adjusted empirically, as is customary in adjusting the time of exposure of photographic materials. The curve relating concentration of penicillin with diameters of zones of inhibition is linear from 1·0 unit per ml. to 8·0 units per ml. when plotted on log-log paper. Figures indicating that penicillins of varying degrees of purity are satisfactorily assayed by the proposed method are quoted, the results obtained being compared with those determined by the standard 18-hr., cup-plate method. J. A.

Organic

Chemical Microscopy of Essential Oils. IV. Cassia and Cinnamon Oils. L. W. Greene (*Amer. J. Pharm.*, 1947, **119**, 59–65)—Three kinds of cinnamon oil: (*a*) cassia, (*b*) cinnamon bark, and (*c*) cinnamon leaf oils, were examined with the following alkaline solutions—(I) a saturated solution of sodium hydroxide in absolute alcohol, (II 30 per cent. sodium hydroxide solution, (III) sodium phenate solution (sodium hydroxide dissolved in phenol in equimolecular proportions and diluted with water to form a 20 per cent. solution), (IV) a saturated solution of potassium hydroxide in absolute alcohol, and with the following organic reagents—(V) phenyl hydrazine and (VI) benzidine.

Results—With (I), (*a*) gives a cloudy yellow amorphous precipitate, (*b*) gives crystals after 0·5 hr. at room temperature, and (*c*) gives long needle-like crystals. With (II) (one drop of reagent to one drop of oil on a slide), (*a*) and (*b*) give almost immediately a white or faint yellow crystalline mass, which develops a definite crystalline structure in 1 to 2 hr., and shows strong interference colours in polarised light. With (III), (*a*) and (*b*), in 10 min., form crystals of long slender microscopic needles which in polarised light show strong feathery markings in interference colours, and (*c*) forms no crystals in 1 hr. With (IV), (*a*) and (*b*) give crystals which are weakly anisotropic in polarised light and practically invisible in ordinary light, and (*c*) gives an anisotropic mass without definite crystalline structure. With (V) (one drop added to one drop of oil on a slide), (*a*) and (*b*) yield interlocking needles of crystalline phenylhydrazone, with form similar to that given by (V) with bitter almond oil, but the latter differs by exhibiting strong polarisation colours; (*c*) forms no crystals. With (V) (one drop added to one drop of oil on a slide), (*a*) and (*b*) form immediately a yellow anisotropic mass of crystals of perfect and imperfect diamond-shaped plates. To obtain with (VI) a reaction product suitable for microscopic examination, the amount of reagent used is just enough to wet the tip of a stirring rod. Photomicrographs of a number of the products are reproduced. E. B. D.

Colour Reactions of Some Unsymmetrical Diarylethylenes. D. S. Tarbell and E. G. Lindstrom (*J. Amer. Chem. Soc.*, 1946, **68**, 1930–1932)—It has been shown, mainly by Pfeiffer and Wizinger (*Ann.*, 1928, **461**, 132; *J. prakt. Chem.*, 1939, **154**, 1) that unsymmetrical diarylethylenes, containing electron-donating groups in the aromatic rings, form coloured addition compounds with a variety of substances. The usefulness of these compounds as detecting agents for a number of toxic substances has been studied with particular reference to arsenicals, such as lewisite or ethyldichloroarsine. The colours produced with these last compounds were determined by adding a drop of the liquid arsenical to the solid ethylene. Dianisylpropylene, when mixed with about twenty times its weight of silica gel, gave a detectable pink colour with less than 1 μg. of lewisite when air containing a few micrograms of the arsenical per litre was drawn through the silica gel. The reaction, however, was inhibited by air of high humidity. The following table gives the colours produced by the action of various diarylethylenes and a liquid arsenical. Brief details for the preparation of the last five compounds are given in the original paper.

Compound	Colour produced
1 : 1-*bis*-(4-Methoxyphenyl)-ethylene	Dark red
1 : 1-*bis*-(4-Methoxyphenyl)-propylene	Dark red
1 : 1-*bis*-(4-Methoxyphenyl)-*iso*butene	No colour
1 : 1-*bis*-(4-Methoxyphenyl)-2-phenylethylene	No colour
1 : 1-*bis*-(4-Dimethylaminophenyl)-ethylene	Intense blue-green
1 : 1-*bis*-(4-Dimethylaminophenyl)-propylene	Intense blue-green
1 : 1-*bis*-(4-Dimethylaminophenyl)-*iso*butene	Green
1 : 1-*bis*-(3 : 4-Dimethoxyphenyl)-ethylene	Transient purple
1 : 1-*bis*-(4-Phenoxyphenyl)-ethylene	Orange red
1 : 1-*bis*-(4-Phenoxyphenyl)-propylene	Faint transient pink
1 : 1-*bis*-(4-Methylthiolphenyl)-ethylene	Transient light green
1 : 1-*bis*-(4-Methylthiolphenyl)-propylene	No colour

J. A.

Colorimetric Micro-method for the Determination of Formic Acid. W. M. Grant (*Anal. Chem.*, 1947, **19**, 206–207)—The gravimetric and titrimetric methods for the determination of formic acid, depending upon measurement of the amount of carbon dioxide or mercurous chloride formed in its reaction with mercuric chloride, are not applicable to the determination of amounts of formic acid less than 1 mg. To increase the sensitivity of the mercurous chloride method a colorimetric procedure has been developed, which permits the determination of amounts of 5 to 30 μg. in 1 ml. with an accuracy of ±1 μg. The method is based upon the chromogenic reduction of a mixture of phosphomolybdic and phosphotungstic acids by the washed mercurous chloride formed in the reaction. Formic acid itself does not give a colour with the reagent.

Procedure—To determine the relationship between the colorimetric readings and the formic acid concentration, prepare a series of dilutions of a standard sodium formate solution (44·4 mg. per litre) containing the equivalent of 0 to 30 μg. of formic acid per ml. To 1 ml. of each solution in a 15-ml., conical centrifuge tube add 0·5 ml. of a reagent containing 20 g. of mercuric chloride, 30 g. of sodium acetate, and 8 g. of sodium chloride in 100 ml. of water, and heat the mixture on the water-bath for 3 hr., preventing evaporation by protecting the upper two-thirds of the tube from excessive heating. After thorough cooling, add 2 ml. of a diatomaceous earth suspension (20 mg. of Dicalite laboratory Filter Aid in 100 ml. of water) to increase the bulk of the precipitate, and to prevent retention of the particles in the surface film add about 10 ml. of alcohol rapidly from a coarse burette tip. Centrifuge the tubes at 2000 r.p.m. for 10 min., after which the liquid can be decanted without loss of precipitate. Wash with two 10- or 15-ml. portions of water by centrifuging and decanting. To each washed precipitate add 1 ml. of the colour reagent. To prepare this dissolve 10 g. of phosphotungstic acid and 130 g. of phosphomolybdic acid in sufficient water at room temperature to yield 400 ml., then add 50 ml. of concentrated hydrochloric acid and 50 ml. of 85 per cent. phosphoric acid. Although a slight sediment forms, the supernatant light yellow solution is stable for several weeks in a stoppered Pyrex bottle. The gradual appearance of a green tinge is accompanied by a slight increase in the colour of the blank determination. Stir the precipitate by striking the tube with the finger and heat on the steam-bath for 15 min. Without cooling the tube, add 4 ml. of saturated sodium carbonate solution and measure the blue colour photo-electrically, plotting the galvanometer readings against the concentrations of formic acid. Residual diatomaceous earth does not interfere significantly with the colour measurement, there is no appreciable change in colour density in at least 10 min., and no turbidity appears when the mercurous chloride precipitate has been adequately washed.

Unknown samples to which the mercuric chloride method can be directly applied are treated in the same manner as the standard solutions and the formic acid concentration is determined by comparison of the colour density measurements with the standard values.

Formic acid in biological material (*e.g.*, blood) is first separated from interfering substances by protein precipitation and low-temperature distillation under reduced pressure in the presence of a strong acid. With blood, it was found satisfactory to centrifuge a mixture of 1 ml. of blood with 1 ml. of water and 1 ml. of 10 per cent. sulphosalicylic acid solution, then to add about 0·02 ml. of sulphuric acid to the separated supernatant liquid and to distil this to dryness at room temperature. This distillation can be conveniently made in an appropriate evacuated and sealed tube (Grant, *Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 729) with the condenser cooled in a bath of dry ice. Recoveries of formic acid added to blood averaged 98 per cent. with an absolute deviation from the mean of 5·1 per cent.

At low concentrations of formic acid, there is a slight deviation from Beer's law, probably due to loss of some mercurous chloride in the washing water, and a slight scattering of the readings round the curve is probably due to mechanical losses. These combined errors amount to about ±1 μg. and are not diminished by reduction of the scale of the method, although slight improvement is obtained by the use of washing water saturated with mercurous chloride.

Samples to be analysed must be free from other reducing substances, such as formaldehyde and ascorbic acid. A. O. J.

Disintegration of Viscose (in the Analysis of Paper Fibres). B. L. Browning and J. H. Graff (*Paper Trade J.*, 1947, **124**, No. 9, Feb. 27, 134)—Papers treated with viscose are difficult to disintegrate by the usual procedures of fibre analysis, and the following method is recommended. Place the sample, torn into small pieces, in a beaker containing 50 per cent. calcium nitrate solution, and heat just below the b p. When the paper starts to disintegrate, remove it, rinse it in hot water, and disintegrate it further by boiling it in 0·5 per cent. sodium hydroxide solution. Wash it successively with distilled water, cold 0·2 per cent. hydrochloric acid solution, and water again, and defibre the paper finally by rolling it into pellets and shaking these with water in a conical flask. The usual staining methods are unaffected by this procedure. The method is unsuitable for paper that has been treated with synthetic resins to produce wet strength. J. G.

Identification of Melamine. E. J. Candlin (*J. Soc. Dyers and Colourists*, 1947, **63**, 144)—Published methods having proved unsatisfactory, the formation of a picrate was investigated. A yellow precipitate is obtained on mixing a neutral melamine solution with concentrated, aqueous picric acid solution; it melts at 312° to 315° C. (uncorr.) after recrystallisation from water. Since formaldehyde inhibits its formation, a method of simultaneous hydrolysis and oxidation is used when melamine - formaldehyde compounds are being examined.

Procedure—Add 5 g. of melamine - formaldehyde condensate to 250 ml. of dilute sulphuric acid and heat to 70° C. Add solid sodium permanganate to decolorise the solution, and filter off carbonaceous matter and manganese oxides. Decolorise the solution with sulphur dioxide, add an excess of sodium hydroxide to precipitate dissolved manganese, and boil. Filter again, neutralise the filtrate with sulphuric acid, and add an excess of saturated, aqueous picric acid. The derivative forms rapidly.

As potassium and ammonium ions form explosive picrates, they must be excluded from the solution. The following oxidising mixtures were unsuitable: sodium hydroxide and hydrogen peroxide, sodium chromate in acid solution, sodium persulphate in hydrochloric acid, and manganese dioxide in hydrochloric acid.

Satisfactory qualitative results have been obtained on melamine - formaldehyde condensates and on fabrics treated with this type of product, but, as much of the resin originally present may disappear during hydrolysis and oxidation, only positive tests may be safely regarded as conclusive.

M. E. D.

Inorganic

Ferrous Ethylenediamine Sulphate as an Oxidimetric Standard. K. P. Caraway and R. E. Oesper (*J. Chem. Educ.*, 1947, **24**, 235–236)—Ferrous ethylenediamine sulphate $(CH_2NH_2)_2SO_4.FeSO_4.4H_2O$, is easily prepared, purified, and dried, and has been shown to be unoxidised after 149 days' standing in air; it is neither deliquescent nor efflorescent.

Preparation—Add 60 ml. of 6 *N* sulphuric acid to 10 g. of 98 per cent. ethylenediamine solution. Add 46·3 g. of ferrous sulphate and, when dissolution is complete, dilute to 300 ml. with water. Stir with 300 ml. of ethanol and filter on a Buchner funnel, washing with 50 per cent. ethanol. Dissolve the precipitate in acidified water, and add two-thirds of the volume of alcohol to reprecipitate the salt. Filter again, and wash with 65 per cent., and finally 95 per cent., ethanol; dry at 50° C (*cf.* Grossman and Schuck, *Z. anorg. Chem.*, 1906, **50**, 26).

Use as a standard—To standardise a 0·05 *N* oxidising solution, dissolve 0·4 g. of ferrous ethylenediamine sulphate in 150 ml. of water and 10 ml. of diluted sulphuric acid (1 + 1). When using dichromate, add also 5 ml. of diluted phosphoric acid (1 + 1). Titrate then with the oxidising solution using an appropriate indicator.

The salt has been used to standardise ceric sulphate, potassium permanganate, and potassium dichromate solutions and, in the range of 0·02 to 0·2 *N* solution, the results agree well with those given by the usual methods.

Ceric sulphate solution has been standardised against (i) ferrous ethylenediamine sulphate, (ii) sodium oxalate, and (iii) potassium dichromate through sodium thiosulphate, and the 9 values given were all either 0·0499 or 0·0500 *N*. M. E. D.

Use of Cacotheline in Volumetric Analysis. I. Determination of Calcium Salts by Titration with Sodium Oxalate. II. Determination of Tervalent Iron by Titration with Stannous Chloride Solution. M. L. Kutschment and A. I. Gengrinovitsch (*Zavod. Lab.*, 1945, **11**, 267–269)—*Determination of calcium*—Hitherto no direct method of titrating calcium has been described. It is now shown that cacotheline may be used as an indicator in presence of ferrous sulphate with sodium oxalate as titrating solution. When all the calcium has been precipitated, sodium oxalate starts to combine with tervalent iron and favours the reduction of cacotheline by the ferrous salt. The end-point is the appearance of a violet colour.

Procedure—To the solution of the calcium salt, neutral or acid with acetic acid, add 1 ml. of a saturated aqueous solution of cacotheline, and one drop of a saturated solution of ferrous sulphate, and titrate with 0·1 *N* sodium oxalate until a violet colour appears. The reaction is rapid and the titration takes only 1 to 2 min. The colour is stable for several minutes. In acetic acid solution, magnesium salts may be present to at least five times the quantity of calcium. Results are accurate to about 1 part in 300.

Determination of iron—Cacotheline, present in a ferric solution which is titrated with stannous chloride solution, is not reduced until all the iron is in the ferrous state.

Procedure—To the solution of ferric iron in 2 *N* hydrochloric acid add 1 ml. of cacotheline solution, heat to boiling, and titrate with standardised stannous chloride solution to the appearance of a violet colour. Prepare the stannous chloride solution by dissolving 30 g. of stannous chloride in a mixture of 300 ml. of concentrated hydrochloric acid and 700 ml. of water by boiling, and establish the titre on potassium iodate in presence of starch (*cf.* Korenman, "*Quantitative Microchemical Analysis*," 1936, p. 143). The end-point is very sharp. Results are accurate to 2 or 3 parts in 1000.

G. S. S.

Determination of Traces of Copper by Extractive Dithizone Titration. C. H. Schonk (*Chem. Weekblad*, 1946, **42**, 295–301)—The speed of reaction of copper with dithizone solution depends on the *p*H value, increasing with an increase in the *p*H. A quick reaction is desirable, as it makes the titration sharper. On the other hand, increasing the *p*H results in interference by zinc, so that a *p*H of 2·8 represents an optimum value for this determination in simple aqueous solution. In presence of large concentrations of salts, the speed of reaction decreases above *p*H 2·8, but the interference by zinc is also less, so that for such solutions a *p*H of 3·3 is chosen. The indicator used for adjustment of the solutions is the potassium salt of benzenesulphonic acid azo-benzylaniline. The reaction is also accelerated by various impurities, and for this reason the dithizone is not subjected to a purification process. Chromium also accelerates the reaction. Since the reaction is an equilibrium one, the titration is not directly proportional to the copper present, as shown by the following table:

µg. Cu	ml. dithizone
1	1·0
2	1·8
4	3·4

Thus, the titre of the solution must be determined against a standard copper solution of a similar concentration and composition to that of the unknown. The amount of zinc present should not exceed 500 µg. per 100 ml. for water solutions and 100 µg. for solutions prepared by wet destruction. Interference by mercuric and silver ions may be prevented by addition of 0·3 ml. of a 50 per cent. solution of potassium iodide. Bismuth reacts after copper, and does not interfere if standardisation is carried out on the titrated solution. Stannous salts are oxidised by hydrogen peroxide, the latter being removed by sulphite. Ferric iron should not exceed 60 µg. for water solutions, or 2000 µg. for solutions prepared by wet oxidation. Larger quantities may be masked by sodium fluoride, of which 10 mg. are sufficient for 1·5 mg. of iron. If organic matter is destroyed by wet oxidation with sulphuric and nitric acids, it is important to remove all nitric acid.

Special reagents, other than copper-free water, acids, etc., are as follows—*Dithizone solution:* a solution of the commercial compound in carbon tetrachloride containing about 2 mg. per 100 ml. About 3 ml. should be used in the titration. This solution is kept in a separating funnel under a layer of a solution of sodium sulphite in 2 *N* sulphuric acid. *Chromium solution:* a 20 per cent. solution of $Cr_2(SO_4)_3,18H_2O$ with a few drops of sulphuric acid, freed from copper by extraction with dithizone, and then washed with carbon tetrachloride.

Procedure—To 100 ml. of the solution, in a separating funnel, add any masking reagents, and a few drops of indicator, and adjust the *p*H to 2·8 or 3·3 (see above). Add three drops of chromium solution (this is unnecessary after wet combustion), and add the dithizone solution from a micro-burette, at first in 0·4-ml. portions, and finally in 0·1-ml. portions. After each addition shake vigorously for 45 sec., and allow to settle. Before each successive portion is added the preceding portion is run off. Continue the titration until the tetrachloride layer has a distinct green colour. At this point there is an excess of dithizone, and to correct for this only the half of the last portion added is reckoned in the measurement. If the amount of copper is very small, the liquid should first be saturated with carbon tetrachloride. After the completion of the titration, wash the liquid with a small amount of the tetrachloride, add a quantity of standard copper solution approximately equal to that found, and carry out another titration in order to standardise the dithizone solution under the same conditions as those of the original titration. G. M.

Determination of Available Chlorine [in Paper]. W. H. Birchard (*Pulp and Paper Mag. Canada*, 1947, **48**, Feb., 62)—Existing methods (*e.g.*, *T.A.P.P.I.* Standard T.611 m–45) in which the difference between the total chlorides, determined by oxidation with hydrogen peroxide and titration with standard silver nitrate, and the available chlorine, determined by titration, with standard sodium thiosulphate, of the iodine liberated from potassium iodide solution in presence of acetic acid, is taken to give the chlorides present, actually only indicate the amount of chloride present in excess of the chloride formed (as calcium chloride) when calcium hypochlorite is formed by passing chlorine into lime water. A similar error exists with the arsenious acid method. They arise from the convention of expressing the results in terms of bleaching powder containing 35 per cent. of chlorine. The methods should be revised so as to give the result in terms of chlorine available for bleaching, *i.e.*, of the hypochlorite content. J. G.

Analysis of Mixtures of Mercurous and Mercuric Mercury and Sulphuric Acid. B. Warshowsky and P. J. Elving (*Anal. Chem.*, 1947, **19**, 112–114)—In order to find the efficiency of a used mercuric sulphate catalyst solution, the total mercury is determined by titration with standard thiocyanate after oxidising the reduced mercury present with ceric sulphate. Mercurous salts are determined separately by oxidation to the mercuric iodide complex with iodine liberated from an excess of standard iodate solution, and determination of the excess of iodine. The mercuric content is determined by difference. The sulphuric acid is titrated with standard alkali in presence of an excess of sodium chloride to prevent buffering by hydroxy-mercuric compounds, and to precipitate mercurous chloride.

Procedure—Total mercury—Treat a sample of the catalyst solution containing 0·1 g. of mercury salts with 100 ml. of water, 20 ml. of diluted sulphuric acid (1 + 1), and 50 ml. of 0·1 *N* ceric sulphate containing 30 ml. of concentrated sulphuric acid per litre. Oxidise the mercury by boiling for 20 to 30 min., cool, and add 3 drops of *o*-phenanthroline ferrous indicator. From a burette add 0·2 *N* ferrous sulphate solution (39 g. of ferrous ammonium sulphate crystals diluted to 500 ml. with 0·1 *N* sulphuric acid) almost to the equivalence point to remove the excess of ceric ions, but leaving the solution coloured slightly green. Add 2 ml. of 40 per cent. ferric sulphate solution and titrate with 0·1 *N* potassium thiocyanate between 15° and 20° C. A blank correction is unnecessary, and any precipitate of mercuric thiocyanate formed near the end-point can be disregarded.

Mercurous mercury—Treat a sample containing approximately 0·25 g. of mercurous mercury with 25 ml. of 0·1 *N* potassium iodate, 1 g. of potassium iodide dissolved in a few millilitres of water, and 10 ml. of *N* sulphuric acid, and stopper immediately. Shake to dissolve the precipitate first formed, and then rinse the stopper and titrate the washings and solution with 0·1 *N* thiosulphate, in presence of starch.

Sulphuric acid—Add 10 ml. of 10 per cent. sodium chloride solution to a sample containing 0·25 g. of sulphuric acid and dilute to 150 ml. Titrate with 0·1 *N* sodium hydroxide in presence of methyl red. M. E. D.

Determination of Aluminium in High Alloy Steel. E. I. Fogelson and N. V. Kalmikova (*Zavod. Lab.*, 1945, **11**, 359–360)—After removal of silicon, and electrolysis with a mercury cathode, manganese is precipitated by means of persulphate, and titanium, vanadium, and iron in solution are thrown down by means of cupferron. Adjustment of the *p*H then gives a precipitate of aluminium as its cupferron complex, free from other metals. The method is claimed to be quicker than the usual methods, such as the 8-hydroxyquinoline method, and to give an uncontaminated precipitate.

Procedure—Dissolve 0·5 g. of sample by heating with 40 ml. of diluted sulphuric acid (1 + 3), oxidise with nitric acid, evaporate to fuming, dissolve the residue in water, and filter. Ignite the insoluble silica, volatilise it with hydrofluoric acid, fuse the residue with potassium pyrosulphate, dissolve the melt in water and return it to the main solution. Electrolyse the solution as usual, using a mercury cathode, then heat the electrolyte, preferably after evaporation to a volume of 100 ml., with 2 g. of ammonium persulphate until manganese is completely precipitated, and filter. Add to the filtrate 8 ml. of concentrated sulphuric acid, dilute with water to 200 ml., and add 2 per cent. cupferron solution to precipitate titanium, vanadium, and iron. Filter and neutralise the filtrate carefully, after making it very cold and stirring, with ammonia in presence of Congo red indicator, and add 20 ml. of buffer mixture to give a *p*H of 4 to 5. The buffer mixture is prepared by neutralising 6 ml. of glacial acetic acid with aqueous ammonia to litmus, adding 3 ml. of glacial acetic acid, and diluting with water to 100 ml. Normally the excess of cupferron remaining in the solution after precipitation of titanium, etc., is sufficient for the precipitation of aluminium, but with large amounts of aluminium, add a further 3 to 5 ml. of cupferron solution. Filter and ignite preferably as described by Zvenigorodskaja and Tschernikov (*Zavod. Lab.*, 1940, 9, 1089). G. S. S.

Colorimetric Determination of Iron in Brass and Bronze. W. Goodman (*Anal. Chem.*, 1947, **19**, 141–142)—Hitherto the failure of colorimetric methods for this determination has been due to loss of iron by co-precipitation in separating the other constituents. This is avoided by direct determination in solution.

Procedure—Treat a 1-g. sample in a 200-ml. iron-free beaker (Vicor) with 15 ml. of water, 0·5 to 1·0 ml. of 48 per cent. hydrofluoric acid, and 10 ml. of nitric acid (sp.gr. 1·42). Cover with a platinum lid, set aside until dissolution is complete, and then boil to expel oxides of nitrogen. Rinse the lid with water, and dilute the solution and washings to 135 ml. Add 1 drop of 0·1 N hydrochloric acid, and deposit lead and copper by electrolysis at a current of 2 amperes. Reserve the electrolyte for the iron determination.

Dilute the solution accurately to 150 ml. and pipette 1 ml. into a colorimetric tube. Add 18 ml. of hydroxylamine hydrochloride solution (4 g. of the salt, 50 g. of ammonium acetate and 35 ml. of concentrated hydrochloric acid to 1 litre, adjusted to *p*H 4·3 with the acetate or acid. Add 6 ml. of 0·15 per cent. *o*-phenanthroline solution, stopper, and shake thoroughly. Determine the percentage transmittancy of the orange coloration by means of a Fisher electrophotometer, using a No. 425 dark blue filter, adjusted to 100 per cent. transmittancy with distilled water treated as above. Determine the iron content by reference to a calibration curve of percentage transmittancy plotted against iron concentration on semi-logarithmic paper. This curve serves also as a blank determination for the reagents and vessels used.

Results—Using National Bureau of Standards samples ranging from 0·03 to 0·83 per cent. of iron, the average results obtained agreed closely with the certified values.

The presence of fluoride ion does not interfere at *p*H 4·1. Interference due to zinc is avoided by using an excess of *o*-phenanthroline. Aluminium and manganese in amounts up to 250 times the iron concentration, and nickel up to 1 per cent., do not interfere with the method. Iron added to samples containing 4·8 and 9·7 per cent. of tin was quantitatively recovered. M. E. D.

Analysis of Cassiterite. T. L. Pokrovskaja (*Zavod. Lab.*, 1945, **11**, 363–364)—The determination of silica, titania, niobium and tantalum pentoxides, ferric oxide, zirconia, calcium, magnesium, manganese, tungsten, arsenic, heavy metals, and tin is outlined and improved procedures are detailed.

Procedure—The sample (2 to 5 g.) of cassiterite is reduced in hydrogen and tin is volatilised as stannic chloride (this is normal Russian practice). Heat the dry residue with concentrated hydrochloric acid and a few drops of bromine water, evaporate to dryness to remove bromine, and again treat with concentrated hydrochloric acid. Dilute with water, leave the solution just acid after adding aqueous ammonia, add 3 g. of ammonium chloride for each 100 ml. of solution, and heat to boiling. To precipitate niobium, tantalum, titanium, zirconium, and residual tin, add 20 per cent. aqueous pyridine solution until a definite odour of pyridine persists, and then add an excess of 15 to 20 ml. Heat to boiling and allow the solution to stand on a water-bath for 30 to 40 min. to coagulate the precipitate. The solution contains only calcium, magnesium, and manganese, which are determined by normal methods. The advantage of the pyridine precipitation over the normal method is that the earth acids, tin, titanium, zirconium, iron, etc., are all precipitated together and are not distributed between a precipitate and a solution. Filter off the precipitate, wash with 3 per cent. ammonium chloride solution, containing a few drops of pyridine, ignite in a quartz crucible and fuse with potassium pyrosulphate. Dissolve the melt in 20 per cent. tartaric acid solution, filter off the silica (ignite, weigh, treat with hydrofluoric acid, etc., as usual), and pass hydrogen sulphide through the filtrate to precipitate residual tin and other heavy metals. Filter and reject the insoluble material (heavy metals are best determined on a separate sample). Add ammonia to precipitate ferrous sulphide, and then filter again. Determine iron by dissolving the sulphide in 15 ml. of concentrated hydrochloric acid, with a few drops of nitric acid added after solution, and weighing as Fe_2O_3 or determining colorimetrically with sulphosalicylic acid (Nikitina, *Zavod. Lab.*, 1940, 9, 629). Add to the filtrate from the ferrous sulphide sufficient hydrochloric or sulphuric acid to give a 10 per cent. acid content, boil off hydrogen sulphide, and then add 6 per cent. cupferron solution to precipitate the earth acids, titanium, and zirconium. Filter and wash the insoluble matter with 1 per cent. hydrochloric or sulphuric acid solution, containing cupferron. Carefully dry the precipitate, ignite, and weigh; then fuse with pyrosulphate and treat the melt with 1 per cent. tannin in 10 per cent. hydrochloric acid. Boil the solution, filter, ignite, and weigh the earth acids, make the filtrate 10 per cent. in hydrochloric acid, cool, and precipitate zirconium and titanium by means of 6 per cent. cupferron solution. Filter off the precipitate, ignite, weigh, fuse with pyrosulphate, and determine titanium colorimetrically with hydrogen peroxide, and zirconium as phosphate.

On a separate portion determine tungsten, arsenic, and tin by first fusing with sodium peroxide. Acidify the alkaline solution with hydrochloric acid, making the acid content 50 per cent., and precipitate arsenic by means of sodium hypophosphite. Filter and determine arsenic iodimetrically. Determine tungsten colorimetrically with stannous chloride, and tin by the normal iodimetric method.

On a further separate portion carry out the reduction and the removal of tin as in the main method, treat the residue with concentrated hydrochloric acid, filter off the insoluble matter, and precipitate heavy metals from the filtrate by means of hydrogen sulphide, and determine them by the usual methods. G. S. S.

Gas Analysis

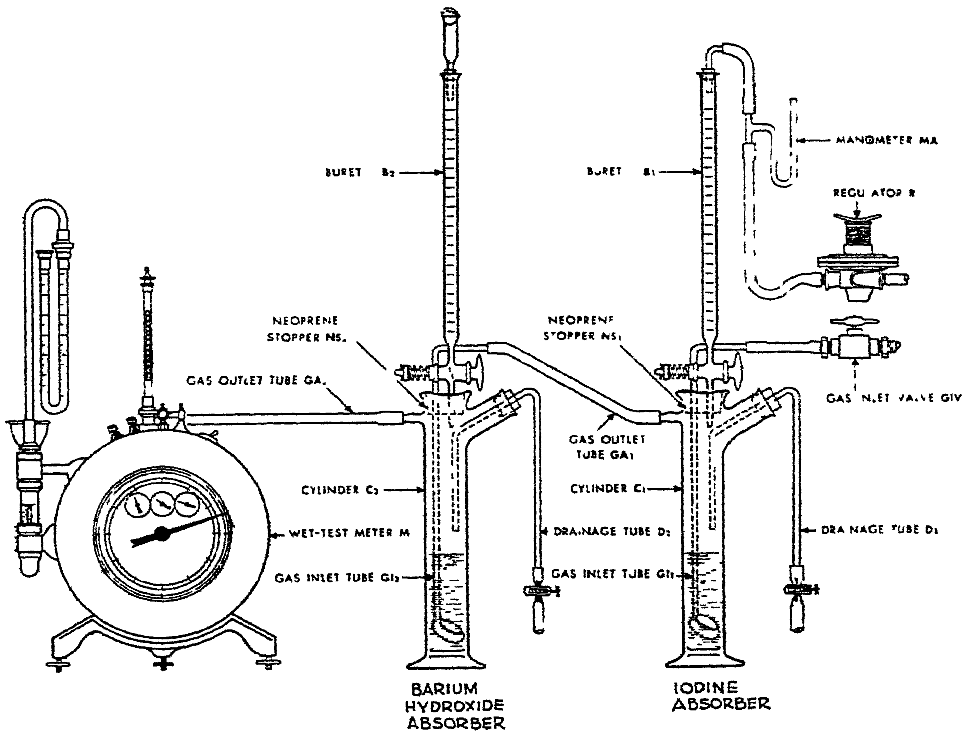
Simultaneous Determination of Hydrogen Sulphide and Carbon Dioxide in a Continuous Gas Stream. C. L. Blohm and F. C. Riesenfeld (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 373–376)—The method is based on the reactions

$$H_2S + I_2 \rightarrow 2HI + S$$

$$Ba(OH)_2 + CO_2 \rightarrow BaCO_3 + H_2O$$

The apparatus is shown in the figure. The absorption vessels are 500-ml. Pyrex hydrometer jars, modified by addition of side arms. All the stoppers and flexible tubing in contact with the gas are of neoprene, as rubber absorbs some hydrogen sulphide. The gas inlet tubes are fitted with sintered-glass dispersion discs. These should be of coarse porosity to avoid blocking of the pores by the precipitated sulphur and barium carbonate. The iodine solution in burette B_1 is kept under a pressure of air or neutral gas of about 150 to 250 mm. of mercury to overcome the pressure in the apparatus.

Method—Put in vessel C_1 150 ml. of water free from carbon dioxide, 5 ml. of 0·4 per cent. starch solution, and enough 0·1 *N* iodine to give a slight but permanent blue colour. Put in vessel C_2 150 ml. of carbon-dioxide-free water and 5 ml. of 0·1 per cent. phenolphthalein solution. Add from the burette B_1 a known volume of 0·1 *N* iodine and from burette B_2 a known volume of 0·25 *N* barium hydroxide. Pass the gas through the apparatus at a rate of 1 to 2 cubic feet per hour, and record the gas meter readings at which the indicators change colour. Add further titration fluids and repeat the procedure. In special cases, use titrating fluids of other concentrations. Iodine from 0·01 *N* to 0·5 *N* and barium hydroxide from 0·1 *N* to 0·5 *N* are suitable. The first readings of a series are discarded as some of the gas is used to saturate the absorbing liquids. Correct the volume of the gas as indicated on the meter for temperature, barometric pressure, water vapour pressure, the volume of the absorbed gases, and the



volume of gas displaced by the addition of titrating fluids.

1 ml. of 0·1 N iodine ≡ 1·183 ml. of hydrogen sulphide at 60° F. and 30 in. of mercury.

1 ml. of 0·1 N barium hydroxide ≡ 1·183 ml. of carbon dioxide at 60° F. and 30 in. of mercury.

Tests of the method on natural gas indicate that the variations between successive determinations do not exceed 2·5 per cent. The method does not discriminate between hydrogen sulphide and mercaptans or other reducing gases, or between carbon dioxide and other acidic gases. L. A. D.

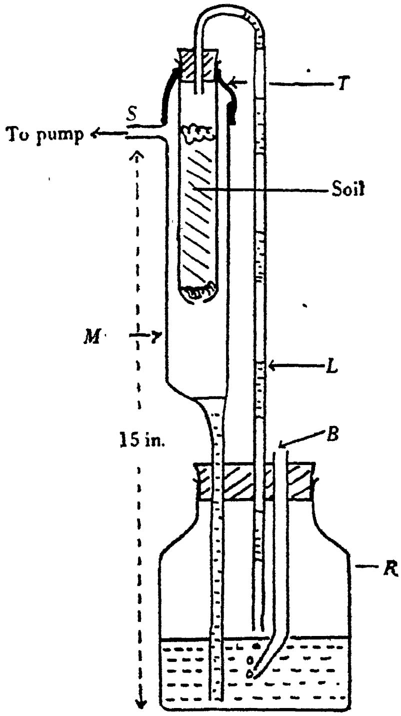
Water Analysis

Determination of Dissolved Oxygen in Presence of Sulphite. C. Janssen (*Chem. Weekblad*, 1946, **42**, 115–119)—In the original Winkler method for the determination of dissolved oxygen in water, the presence of sulphite interferes for two reasons. Iodine reacts with the sulphite in acid solution, and oxygen is used up, not as previously supposed by direct oxidation of the sulphite by the manganous acid formed, but by the catalytic action of the manganese on the reaction between oxygen and sulphite. By the modified manganese carbonate method (Winkler, *Z. anal. Chem.*, 1905, **53**, 665; Bruhns, *Chem. Ztg.*, 1915, **39**, 845; 1916, **40**, 45, 71, 985, 1011; Goldina, *Ber. allruss. warmetechn. Inst. No.* 6, 1934, 43; *Chem. Zentr.*, 1935, II, 2102) low values are also obtained. The method of Haase (*Z. anal. Chem.*, 1937, **90**, 241) employs iodine to remove the sulphite and thiosulphate to remove excess of iodine, but results are also low owing to rapid oxidation of the tetrathionate. Correct results may be obtained by the original method of Winkler if a correction is applied for the sulphite present. Two bottles are filled with the water. In one, determine the oxygen content according to the ordinary Winkler method. In the other, determine the sulphite content by a direct titration of the water with iodine and not by adding an excess of iodine and titrating back. G. M.

Physical Methods, Apparatus, etc.

Operating Characteristics of the Sargent Model XX Visible Recording Polarograph. J. J. Lingane (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 734–738)—The performance of this American instrument is compared with that of other American recording polarographs, and most of the criteria mentioned can be applied to British instruments. The instrument should be robust and immune to vibrations, and adequate electrode assembly and cells should be provided. A continuously variable sensitivity control and a visible recording system are advantageous, and it should be possible to record diffusion currents as a function of time at constant applied e.m.f. The observed diffusion currents and half-wave potentials must be independent of the degree of damping used, and the current sensitivity must be constant. J. G. W.

Simple Automatic Percolator. H. Lees (*J. Agric. Sci.*, 1947, **37**, 27–28)—The apparatus enables automatic percolation of an aerated solution, and changes due to passage through the column may be determined by analyses of the solution returned to the reservoir. It is suitable for soils, bacteria or fungi cultures on porous media, extractions from solids, and some types of chromatography; the gas used may also be varied by using a closed-system pump.



Place 10 to 20 g. of the solid material between plugs of glasswool in a 6 × ¾ in. Pyrex test tube, with a hole in the bottom. T is a piece of 1¼-in. bicycle inner tube. The outer tube, M, is a 8 × 1¼-in. Pyrex tube, fused to a piece of 5-mm. internal diameter Pyrex tubing, and 15 in. above its join is a side-arm, S. Through the bung of the reservoir, R, passes a bubbler, B, and above its submerged end the lift tube, L, which is held in the end of the soil tube by a bung. Apply slight suction at S and bubbles of air and liquid pass up L; the air is drawn off at S and the liquid is returned into M. Isolate the system from the pump, and pipette samples from the reservoir for analysis. Waterlogging is impossible as the decrease of pressure in M lowers the liquid level in R below the end of *L* and percolation stops. The depth of the end of *L* below the liquid surface does not affect percolation, though it affects the head of liquid in *M*. M. E. D.

Precise Low-Pressure Measurements with a Thermocouple Gauge. W. G. Smiley (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 800–801)—Pressure gauges that depend on thermal conductivity (Pirani and thermocouple gauges) are usually operated either at constant current or at constant voltage, when the upper limit of the sensitivity range is due to the temperature coefficient becoming too small for measurement. By operating at constant temperature (constant resistance on the Pirani gauge, constant output on the thermocouple gauge), the range may be considerably extended and precise measurements may be made.

The heater current of a General Electric Type J-1 vacuum gauge was adjusted to give a constant thermal e.m.f. of 3·050 millivolts. The necessary heater current, which is a measure of the pressure, was determined to ± 0·01 milliamp. by measuring the voltage drop across a 0·1 ohm precision resistor. The current varied from 27 milliamp. (below 0·001 mm.) to 150 milliamp. (above 20 mm.). In any one run the gauge was sensitive to a 1 per cent. change in pressure from 0·002 to 20 mm., or to a 0·1 per cent. (or less) change from 0·02 to 2 mm. Since readings were found to vary between runs, the gauge was enclosed in a constant-temperature water-jacket.

The curved characteristic of a thermal pressure gauge necessitates extensive calibration. For measuring the pressure of water vapour above hydrates, the vapour pressure of ice, which is known to within ± 0·2 per cent., is suggested as a convenient standard. J. T. S.

Vacuum Micromanometer. W. S. Young and R. C. Taylor (*Anal. Chem.*, 1947, **19**, 133–135)—Details are given of the construction and calibration of a manometer suitable for measuring pressure in the range 10^{-1} to 10^{-3} mm. of mercury. The pressure is measured by observing the position of a bubble of gas in a capillary tube connecting two large reservoirs of pentane. Pressure changes are transmitted to the pentane by two barometric legs of mercury. The head of one mercury column is in a high vacuum and the gas, the pressure of which is to be measured, is placed in contact with the head of the other mercury column. Differences between pressure measurements made on the same samples of air with the micromanometer and with a McLeod gauge, in the pressure range 10^{-2} to 10^{-1} mm. were not more than $\pm 4 \times 10^{-3}$ mm. and averaged less than $\pm 10^{-3}$ mm. The use of the manometer for molecular weight determinations is described in another paper (*see the next abstract*). B. A.

Molecular Weight Determination with a Vacuum Micromanometer. W. S. Young and R. C. Taylor (*Anal. Chem.*, 1947, **19**, 135–137)—To determine the molecular weight of a liquid, small quantities are allowed to vaporise at room temperature into a large bulb, previously evacuated, and the resultant pressures are measured by a micromanometer of the type described in the previous paper (*Idem.*, *Ibid.*, p. 133; see preceding abstract). The liquid is introduced into the evacuated bulb through a mercury-sealed, sintered-glass disc, F porosity, which is porous to most liquids, but not to mercury. A micro-burette suitable for measuring the liquid sample is prepared from thermometer tubing selected to deliver about 2×10^{-4} ml. per cm. and having 20 to 30 graduations per cm. length. One end is ground to a truncated cone to ensure good contact between the capillary and the sintered disc.

The molecular weights of many organic liquids can be determined with an average accuracy of about ± 2 per cent. Liquids to which the method cannot be applied are those that react with mercury, or have vapour pressures below 0·1 mm. of mercury at room temperature. B. A.

Apparatus for Determining Boiling Points. L. M. Simmons (*J. Chem. Educ.*, 1947, **24**, 233–234)—The apparatus is a glass U-tube of 7-mm. bore with limbs 15 cm. and 9 cm. long. The shorter limb can be closed by a ground plug and the joint sealed by mercury in a mercury cup. Both limbs are graduated in millimetres from a datum line near the bend.

Procedure—Add mercury to reach half way up the scale on the shorter limb, exert pressure to push the mercury to within 2 mm. of the ground socket, and pour about 0·3 ml. of the sample on to the mercury surface beneath the socket. Remove any air bubbles, insert the stopper, and release the pressure. From the barometric pressure and the levels of the mercury in the two limbs calculate the position of the mercury level in the longer limb at which the pressure of the vapour would be 760 mm. Place the tube vertically in a bath of a suitable liquid, heat the bath slowly with stirring, and note the temperature at which the mercury is at the calculated level. Take a further reading as the temperature falls and repeat until good concordance is obtained.

As the volume occupied by the vapour is small and there is no distillation of the sample, the method may be applied to liquid mixtures and may be used to test for the presence of dissolved air and other impurities in a liquid. The reproducibility of results is ± 0·1° C. B. A.

Errors in Spectrochemical (Flame) Analysis. A. C. Oertel and H. C. T. Stace (*J. Soc. Chem. Ind.*, 1946, **65**, 350–354)—A statistical analysis has been made of the errors in determinations of the exchangeable cations in soils by means of an air-acetylene flame and a large quartz spectrograph. Even with an elementary technique an average standard error of less than 4 per cent. was obtained. Most of the error was due to variations in the photographic plates, and the significance of this in relation to the placing of the standard spectrograms on the plate is discussed. In general, a large quartz spectrograph is not suitable for use with an air-acetylene flame because the very low light-collecting power of the instrument necessitates very long exposures or the use of extremely concentrated solutions. B. A.

Reviews

THE PHARMACOPOEIA OF THE UNITED STATES OF AMERICA. Thirteenth Revision (U.S.P. XIII). Pp. cvii + 957. Mack Publishing Co., Easton Pa. Price 8 dollars.

While, to the exasperation of its Editor, the revised post-war edition of the British Pharmacopoeia, owing to printing difficulties, is still in the press, the results of the thirteenth revision of the United States Pharmacopoeia have become available. The U.S.P. XIII came into force on April 1st, 1947, five years after the publication of the twelfth revision, and its contents reflect a period of intensive medical development that has been the outcome of the scientific research stimulated by the second world war. The trend of medical opinion in the United States is indicated by the appearance of ninety-five new monographs and the deletion of monographs on one hundred and twelve substances that appeared in the U.S.P. XII. Monographs deleted include those for lactic, mandelic, phosphoric and sulphuric acids, chloroform water, silver proteinate, belladonna root, bismuth subnitrate, carbon tetrachloride, chloramine-T, decoctions, infusions and several extracts (including extract of malt), reduced iron, nux vomica, ergot, colchicum seed, strophanthin, Blaud's pill, several oils and spirits, antimeningococcus and antipneumococcus sera, salol, prepared suet, sulphapyridine and terpin hydrate. The deletions are indeed striking but scarcely more so than the admissions. Among monographs appearing in the U.S.P. for the first time are those for aluminium phosphate gel, anhydrohydroxyprogesterone (for which Ethisterone is the British approved name), antimony sodium thioglycollate, bentonite magma, benzalkonium chloride (a mixture of alkyldimethylbenzyl ammonium chlorides used in aqueous solution as a detergent), carbachol (carbamylcholine chloride), cholera vaccine, desoxycorticosterone acetate, digitoxin, digoxin, diphtheria and tetanus toxoids, helium, methyl testosterone, penicillin salts and preparations, plague vaccine, progesterone, methyl and propyl hydroxybenzoates (under the names of methylparaben and propylparaben respectively), sodium ascorbate injection, sulphamerazine (2-sulphanilamido-4-methyl pyrimidine), testosterone proprionate, soluble thiopentone, vinyl ether and epidemic typhus and yellow fever vaccines. Many of these substances have become well established in British medicine and monographs for such have already appeared in the Addenda of the British Pharmacopoeia and Supplements of the British Pharmaceutical Codex that have been issued during the war years.

It is not only the trend in modern medicine from the use of vegetable drugs and galenicals to the use of isolated standardised constituents and synthetic drugs that is particularly of interest to analysts, but also the trend towards demanding and achieving more specific standards for substances whether of chemical or biological origin. Reference Standards additional to the International Standards set up by the Permanent Commission on Biological Standardisation of the Health Organisation of the League of Nations are available from the U.S.P. authorities to enhance the accuracy of many chemical, biological and microbiological assays.

It is of particular interest to note that U.S.P. Reference Standards are provided and required (*a*) for the determination of diethylstilboestrol (stilboestrol) in capsules, tablets and injections; (*b*) for the determination of epinephrine (adrenaline) in solutions such as the injection, inhalation and liquor; (*c*) for the determination of the mixed melting-point of methyltestosterone isolated in the assay of tablets. For sulfanilamide (sulphanilamide) a Reference Standard is provided, although it is not required for carrying out the examinations and assays described in the monographs for this substance or for the tablets.

An assay for each vitamin present is described in monographs on Hexavitamin capsules and tablets, which are each required to contain vitamin A, 5000 U.S.P. units; vitamin D, 400 U.S.P. units; ascorbic acid, 75 mg.; thiamine (aneurine) hydrochloride, 2 mg.; riboflavine, 3 mg.; nicotinamide, 20 mg. Vitamins A and D are determined biologically, riboflavine and nicotinamide microbiologically, aneurine by the thiochrome method and ascorbic acid by the use of dichlorophenolindophenol, the precise methods being described in a section dealing with general tests, processes and apparatus occupying 260 pages.

The monographs on penicillin salts and preparations are circumscribed by the requirements of the Federal Food and Drug Administration. The calcium and sodium salts must comply with F.D.A. requirements. It is in striking contrast with the high potencies that are now issued by British manufacturers that for oral administration a minimum potency of 300 units per mg. is permitted; for aqueous injection, a minimum potency of 500 units per mg. except for material containing not less than 90 per cent. of penicillin X (penicillin III) when a potency of not less than 350 units per mg. is permitted; for suspensions containing 100,000 to 200,000 units per ml., calcium salt of minimum potency of 750 units per mg. may be used, though for those containing 300,000 units per ml. the minimum potency must be 900 units per mg. Material now issued from British manufacture, for all purposes, is not lower than the highest of the minimum potencies there described. All ingredients of the formulae used for penicillin dental cones, ointments, tablets and lozenges must be approved by the Federal Food and Drug Administration. All who are interested in the analysis of medicinal substances and preparations will find much of interest in noting the standards, tests and assays adopted by the U.S.P. The layout of the monographs facilitates reference, and it is a new departure that the titles are in English with sub-titles in Latin. Structural formulae are set out graphically although not always with typographical elegance. The paper and binding are excellent, in contrast with those available in present-day Britain, but it was surprising to discover that in the review copy pages 725 to 756 inclusive were missing. FRANK HARTLEY

THORPE'S DICTIONARY OF APPLIED CHEMISTRY. Fourth Edition. Vol. VIII. METH - OILS, ESSENTIAL. Pp. 679. London: Longmans Green & Co., Ltd. 1947. Price 80s.

The Editorial Board now responsible for the production of "Thorpe" (*cf.* ANALYST, 1946, **71**, 395) develops in this eighth volume the policy announced in the seventh volume—and they do it well. Of course there are difficulties inevitable in such a production under present-day restrictions, and others due to the time which must elapse before the whole series can be completed. For example: indexing. There was an index to the first six volumes at the end of Vol. VI, and now each volume is separately indexed; this is necessary and most useful, even if inconsistent. On the other hand, an inconsistency that should be eliminated is in methods of giving references: in some articles they are inset as heretofore; in others there is just a number corresponding to a bibliography at the end. These are, however, minor matters. We can, and do, very much welcome some of the new major articles in this volume on very modern topics, *e.g.*, on mineral and X-ray analysis by Dr. F. A. Bannister, on molecular infra-red spectra by Dr. Sutherland and visible and ultra-violet spectra by Dr. Morton, on nomenclature and on chemical literature. With regard to these latter articles it is noted that Dyson's new proposals are not mentioned, and in the section dealing with the literature of analytical chemistry we are indeed suprised that there is no mention of THE ANALYST or of the *Zeitschrift für analytische Chemie*, in both of which is to be found more information on analytical methods than anywhere else.

The article on naphthalene is almost a book in itself; it runs to 175 pages and is in three parts. We miss the name of Professor Wynne as its author, but there is a grateful acknowledgement to him from the new authors. It is a model of completeness and brings the subject up to date; there follow some pages on naphthenic acids, which are now so important in industry.

Another valuable article is on the moth-proofing of textiles; it is useful to have here the chemical equivalents of many trade names. Then there is information on nicotinic acid, its distribution and its determination by microbiological methods as well as by chemical methods. One could go on long drawing attention to all the "meat" to be found in this volume; it is full of good food and information on matters new and old. Whether the reader is an organic chemist, physical chemist, metallurgist, biochemist or analyst or whatever branch of chemistry is required, Thorpe, like our old friend Nanki-Poo, has a contribution to suit our every mood. Whether interested in milk, monel metal, nickel, nitrogen or the I.U.C. nomenclature or what you will, one cannot read Thorpe's contribution without profit even if it is on a familiar topic, and as a pointer to further sources of information it is most useful.

This volume is as invaluable as its predecessors, and can be cordially commended.

H. E. COX

THE CHEMICAL CONSTITUTION OF NATURAL FATS. By T. P. HILDITCH, D.Sc., F.R.I.C., F.R.S. 2nd Edition. Pp. xiii + 554. London: Chapman & Hall, Ltd. 1947. Price 45s. net.

Our knowledge of the fats has been extended widely during the war years, developing generally on the lines that might have been expected from the later peace-time discoveries. In this new edition of his book, Professor Hilditch is able to record a considerable number of fresh analyses showing the nature and proportion of the constituent fatty acids of vegetable and land animal fats, including human fat, improvements in methods of investigating the glycerides of the more liquid and unsaturated fats, and a good deal of fresh information on individual fatty acids; he has responded to the welcome accorded to his earlier discussion of experimental technique by revising and expanding it; and he has provided a welcome index to the set of five separate indexes which deal with subjects, individual fats and waxes, plant families, natural fatty acids, and natural and synthetic glycerides.

The first edition of this work was hailed in many quarters as an outstanding contribution to our knowledge of the subject, an opinion amply confirmed by time. Professor Hilditch again merits our thanks for bringing his work as nearly up-to-date as possible by including all the more important contributions to the subject published before the end of 1945.

K. A. WILLIAMS

LIMIT TESTS FOR IMPURITIES. By F. REIMERS and K. R. GOTTLIEB. Pp. 198. Kopenhagen: Ejnar Munksgaard. 1946. Danish kronen 10·00.

This work represents Volume I of "Contributions from the Danish Pharmacopoeia Commission." Though it is in Danish, the title-page and preface are reproduced in English, and there is an English summary of 27 pages. The work records a thorough investigation of pharmacopoeial limit tests, *i.e.*, those tests in which limits for an impurity are established by means of comparison tests, having as its special object to determine whether these tests do in fact give a reliable basis for determining the permissible limits of impurity. The experiments showed that in most instances the methods previously used must be modified to some extent before they could be considered satisfactory from this aspect. In other instances, even though improvements were made, the final methods were not considered entirely satisfactory.

The work opens with a discussion of the formation of turbidities. It is interesting to note that the solubility of crystals of barium sulphate (and probably of other hard crystals such as those of calcium oxalate) is increased by 90 per cent. when the size of the crystals is reduced to 0·2 μ., whereas with soft crystals like lead iodide (and probably silver chloride) the solubility at 0·4 μ. is only increased by about 2 per cent. Kolthoff has calculated that barium sulphate crystals of diameter 0·04 μ. are about 1000 times more soluble than those of diameter over 1 μ. The significance of this phenomenon for the present application is obvious. Further, crystals of this type will rapidly grow larger at the expense of the smaller ones. Silver chloride, unlike barium sulphate, is thrown out in a colloidal form so that the effect described above does not enter into the precipitation, though coagulation may alter its appearance.

The tests investigated are those for chloride, heavy metals, ammonia, sulphate, calcium, magnesium, arsenic (with hypophosphite), potassium, sodium, nitrate, phosphate, cyanide, iron, other metals and carbonate. An abstract on p. 485 of this issue describes some of the methods recommended.

For chemists interested in tests of this type the book is well worth buying even for its English summary alone, as this gives not only recommended methods, but also a large number of explanatory footnotes. The standard of printing is high, though a stiff paper cover is not ideal for a work to which reference must continually be made. G. MIDDLETON

THE TECHNICAL AND SCIENTIFIC REGISTER

THE Technical Scientific Register is being carefully brought up to date by the Ministry of Labour and National Service, and the Ministry is anxious to make sure that no qualified chemist is omitted from the list. Members who for any reason did not receive the letter, and accompanying questionnaire, which has recently been issued are advised to apply for these to the Technical and Scientific Register, York House, Kingsway, London, W.C.2, or to any Regional Appointments Office of the Ministry and to furnish as fully as possible the information sought.

PURITY OF NICOTINIC ACID USED IN ASSAYS

SAMPLES of nicotinic acid prepared from β-picoline are liable to contain various proportions of *iso*-nicotinic acid. It has been established that *iso*-nicotinic acid is unavailable to *Lactobacillus arabinosus*, but that under certain conditions it may give rise to colour development with various amines. The presence of *iso*-nicotinic acid in any sample of nicotinic acid used as standard may therefore lead to discrepancy between chemical and microbiological results. The purity of the nicotinic acid used as standard is thus as important in microbiological as in chemical assays.

MAINTENANCE OF LABORATORY ANIMAL COLONIES

MEMBERS of the Biological Methods Group, and others interested in the production and care of laboratory animals, may like to know that the New York Academy of Sciences conducted a symposium in November, 1944, on the subject of animal colony maintenance. Nine papers were presented on various aspects of this subject, and these have since been published in the Annals of the Academy, Vol. **46,** 1–126. Copies of the collected papers bound together are available at $1·50 per set.

BIOLOGICAL METHODS GROUP

THE Annual General Meeting of the Biological Methods Group will be held at 6 p.m. on Tuesday, December 16th, 1947, in the Rooms of the Chemical Society, Burlington House, London, W.1.

It will be followed at 6.15 p.m. by an Ordinary Meeting of the Group, at which the following papers will be presented and discussed: "A modified Method for the Microbiological Assay of Tryptophan, Methionine, Cystine and Tyrosine," by Dr. E. C. Barton-Wright and Mr. N. S. Curtis; "The Use of *Neurospora Crassa*, mutant 9185, for the Assay of Aneurine," by Mr. J. S. Harrison and Mr. E. J. Miller; "A Note on the Cup Method of Microbiological Assay and its Limitations," by Dr. W. F. J. Cuthbertson.

The Annual General Meeting will be confined to members of the Group, but guests will be very welcome at the Ordinary Meeting.

REPORTS OF THE ANALYTICAL METHODS COMMITTEE AND OTHER REPRINTS FROM "THE ANALYST" OBTAINABLE THROUGH THE EDITOR

The Reports of the Analytical Methods Committee and other Reprints listed below may be obtained direct from the Editor of THE ANALYST, 7–8, Idol Lane, London, E.C.3 (not through Trade Agents), at the price, except where otherwise stated, of 1s. 6d. to Members of the Society, and 2s. 0d. to non-Members. Remittances must accompany orders and be made payable to "Society of Public Analysts."

ANALYTICAL METHODS COMMITTEE REPORTS

The Reichert-Polenske-Kirschner Process. (Test for Butter Fat.)

Milk Products Sub-Committee:

Reports Nos. 1 and 2. Analysis of Condensed Milks.
Report No. 3. Analysis of Sweetened Condensed Milk in which the Sucrose has altered during Storage.
Report No. 4. Determination of Water, of Total Solids and of Fat in Dried Milk.

Sub-Committee on Dirt in Milk. Report. Determination of Dirt in Milk.

Report on the Determination of Total Solids in Fresh Liquid Milk.

Essential Oil Sub-Committee:

Report No. 1. Estimation of Cineole in Essential Oils. (1) Cajaput and Eucalyptus Oils.
Report No. 2. Physical Constants (1).
Report No. 3. Physical Constants (2).
Report No. 4. Interim Report on the Determination of Acetylisable Constituents in Essential Oils.
Report No. 5. Determination of Phenols in Essential Oils.
Report No. 6. Determination of Citral in Lemon Oil.
Report No. 7. Determination of Solubilities.
Report No. 8. Determination of Cineole in Essential Oils. (2) Camphor Oil. (3) Other Oils.
Report No. 9. Determination of Carvone and Menthone.
Report No. 10. Determination of Citronellal.
Report No. 11. Determination of Aldehydes other than Citronellal.
Report No. 12. Determination of Ascaridole.
Report No. 13. Determination of Esters.
Report No. 14. Solubility Test for Ceylon Citronella Oil. (Gratis.)

Sub-Committee on the Determination of Arsenic, Lead, etc. in Food Colouring Materials:

Report No. 1. Determination of Arsenic.
Report No. 2. Determination of Lead.
Report No. 3. Determination of Copper.

Sub-Committee on the Determination of Unsaponifiable Matter in Oils and Fats and of Unsaponified Fat in Soaps:

Report No. 1. Determination of Unsaponifiable matter in Oils and Fats.
Report No. 2. Determination of Unsaponified Fat in Soap.
Report No. 3. Determination of Free Alkali in Soaps.
Report No. 4. Determination of Free Alkali and Silica in Silicated Soaps.
Report No. 5. Determination of Rosin in Soaps.
Report No. 6. Determination of Phenols in Soaps.

Poisons Sub-Committee appointed to investigate Methods of Assay for Various Substances appearing in the Poisons Schedules of the Poisons Regulations, 1935:

Report No. 1. Assay of Lobelia (*Lobelia Inflata*).
Report No. 2. Assay of Gelsemium
Report No. 3. Assay of Aconite.

Fluorine in Foods Sub-Committee:

Report on the Determination of Fluorine in Foods.
Addendum to above Report. (Gratis.)

Sub-Committee on Vitamin Estimations. Microbiological Panel:

Report on the Microbiological Assay of Riboflavine and Nicotinic Acid.

OTHER REPRINTS

Bibliography of the More Important Heavy Metals occurring in Food and Biological Materials
(For the years 1929 to 1933 inclusive.)
Price 2s. 0d. to Members; 3s. 0d. to non-Members.

Methods of Analysis for the Purposes of the Cake and Flour Confectionery (Control and Maximum Prices) Order, 1942. (S.R. and O., No. 2103 of 1942.)

Lecture by Professor J. Heyrovský on "The Fundamental Laws of Polarography." Delivered at a meeting of the Society on October 31st, 1946.

DECEMBER, 1947 VOL. 72, No. 861

THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

A JOINT meeting of the Society with the Food Group of the Society of Chemical Industry was held at 7 p.m. on Wednesday, December 3rd, 1947, at the Chemical Society's Rooms, Burlington House, Piccadilly, London, W.1. On the invitation of the Chairman of the Food Group, Dr. E. B. Hughes, the Chair was taken by the President of the Society, Mr. Lewis Eynon.

The general subject of the meeting was "The Occurrence, Physiological Importance and Estimation of Metallic Contaminants in Foodstuffs," and the following papers were presented and discussed:—"The Occurrence of Metallic Contaminants in Foodstuffs," by G. E. Forstner, M.Sc., A.R.I.C.; "The Public Health Aspect of Metallic Contaminants in Foodstuffs," by G. W. Monier-Williams, O.B.E., M.C., M.A., Ph.D., F.R.I.C.; "Trace Metals in Human Nutrition," by W. F. J. Cuthbertson, B.Sc., A.R.I.C.; "The Estimation of Metallic Contaminants in Foodstuffs," by N. L. Allport, F.R.I.C., and D. C. Garratt, B.Sc., Ph.D., F.R.I.C.

NEW MEMBERS

Kyle Campbell, B.Sc., F.R.I.C.; Samuel Manoranjan Chanmugan, B.Sc. (Lond.), A.R.I.C.; Edward Watson Clark, B.Sc. (Leeds); Arthur William Hartley, A.M.C.T., A.R.I.C.; Herbert Makin, A.M.C.T.; George Rippon Nellist, F.R.I.C., M.Inst.F.; John Gerard Reynolds, F.R.I.C.; Leslie Singleton; Reginald Smart, B.Sc. (Lond.), A.R.I.C.; Emrys Islwyn Williams, B.Sc. (Lond.), A.R.I.C.; Ernest Raymond Willott, A.R.I.C.

DEATH

WE regret to have to record the death of

Sir Bernard H. Spilsbury, Honorary Member.

PHYSICAL METHODS GROUP

THE Third Annual General Meeting of the Physical Methods Group was held at 6 p.m., on Tuesday, November 25th, 1947, in the Rooms of the Chemical Society, Burlington House, London, W.1. Mr. R. C. Chirnside, the Chairman of the Group, presided.

The Officers and Members of Committee for the forthcoming year are as follows:—

Chairman: Dr. J. G. A. Griffiths.

Vice-Chairman: Mr. B. S. Cooper.

Hon. Secretary: Dr. J. E. Page, Glaxo Laboratories, Ltd., Greenford, Middlesex.

Members of the Group Committee: Dr. W. Cule Davies, Dr. J. R. Edisbury, Dr. W. F. Elvidge, Mr. J. Haslam, Mr. R. A. C. Isbell and Mr. D. M. Smith and, *ex officio*, the President, the Hon. Treasurer and the Hon. Secretary of the Society and the Editor of THE ANALYST.

Mr. C. A. Bassett and Dr. D. C. Garratt were re-appointed Hon. Auditors.

The meeting passed a vote of thanks to the retiring members of Committee, Mr. R. C. Chirnside, Dr. S. Judd Lewis and Dr. F. Wokes, for their services to the Group, and especially thanked Mr. Chirnside for his services as Chairman since the inauguration of the Group.

Following the Annual General Meeting, an Ordinary Meeting of the Group was held, at which Mr. B. S. Cooper delivered a lecture on "Electron Microscopy."

The following three papers on Physical Methods of Gas Analysis were read at a meeting of the Physical Methods Group on May 2nd, 1947, at Newcastle-upon-Tyne.

A Procedure for Gas Analysis at Low Pressures*

By C. E. RANSLEY

THE methods available for the micro-analysis of gas mixtures may be classified broadly into two groups, in which the sample is manipulated (*a*) at approximately atmospheric pressure and (*b*) at very reduced pressures, usually less than 0·1 mm. of mercury. In the first group the analytical procedure usually follows orthodox chemical methods and the constituent gases of the mixture are removed one by one with a series of specific reagents. Various ingenious techniques have been evolved for the handling of very small volumes of gas; most of them involve the use of capillary burettes at constant pressure, and the manipulation of the reagents either as solids or in the form of impregnated glass beads. Suitable apparatus and procedures have been described by Blacet *et alia*[1] and Sutton[2]; a modified apparatus for analysis at constant volume, in which the gas is circulated through reagents contained in loops of capillary glass tubing, has been suggested by Spence.[3] With care, the accuracy of such analysis can be comparable with that obtained in ordinary macro-analysis, but it is difficult to deal with samples smaller than about 0·1 ml.

The feasibility of analysing minute quantities of gas by a relatively simple procedure at low pressures was first demonstrated as early as 1912 by Langmuir,[4] who found such a technique of great value in his work on gas reactions at solid surfaces. In this type of analysis the gases are usually confined in a fixed volume, and the pressure changes resulting from a series of physical or chemical processes are followed by means of a McLeod or other suitable gauge. The sensitivity possible is very much greater than that of the orthodox chemical methods, and it is estimated that 0·001 ml. of gas may be analysed in this way. The great sensitivity is due to the fact that there are high-vacuum gauges available for measuring pressures down to 10^{-5} or 10^{-6} mm. with considerable accuracy, so that a pressure difference corresponding, for example, to 10^{-9} g. of hydrogen can be measured with some precision.

The number of papers published on applications of the low-pressure method is not large. Ryder,[5] in 1918, described an apparatus for dealing with gases evolved from heated metals, and outlined an analytical procedure suitable for the analysis of mixtures containing water vapour, carbon monoxide and dioxide, hydrogen, nitrogen and oxygen. More elaborate apparatus for similar work on gas - metal systems was developed by Norton and Marshall[6] and by Smithells and Ransley.[7] A simplified type of equipment, such as one described by Chipman and Fontana,[8] has been designed for analysing the gases evolved in the vacuum fusion method now widely used for the determination of the total oxygen contents of steels.

In a more recent paper, Prescott and Morrison[9] give details of an apparatus and technique for the rapid analysis of samples with a total volume of 1 to 25×10^{-3} ml. of gas, with a probable error for each component of about 5 per cent. of the total sample. The apparatus is a refined version of one described previously[10] and has been applied by them to problems arising from a study of the behaviour of thermionic cathodes. This procedure can be used for mixtures consisting of water, carbon dioxide, hydrogen, carbon monoxide and oxygen or alternatively methane; and as far as we know, it is the only one published that gives a specific method for the determination of methane.

An important contribution to low-pressure technique was made in 1921 by Campbell,[11] who introduced a method for the identification of gases and vapours by means of their characteristic vapour-pressure curves, which can be determined quite simply with the aid of the Pirani gauge. This is an extremely useful method of analysing mixtures of gases that can be condensed out by liquid oxygen, and some applications have been described by Ransley[12] and Wooten;[13] it has also been used successfully for the analysis of complex hydrocarbon mixtures, by Sebastian and Howard.[14]

Micro gas analysis at low pressures has also been employed for investigating the gases evolved by heated glasses, and contained in "seeds" in glass, and an account of the special technique required for this kind of work will be found in a paper by Shadduck and Van Zee,[15] which should also be consulted for earlier references in this field. The gases evolved from glass usually contain a high proportion of condensable gases such as carbon dioxide and sulphur dioxide, and the Campbell method of low-temperature distillation can therefore be used with considerable advantage in such investigations.

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* Contribution from The Research Laboratories of The British Aluminium Co., Ltd., Chalfont Park, Gerrard's Cross, Bucks.

The apparatus described in the present paper has been developed primarily for work on gases in metals, and is used for the measurement and analysis of the gases evolved from heated aluminium. The gas-content of aluminium has an important influence on the properties of both cast and wrought products, and we have recently been investigating the extraction of gases from the heated metal in both solid and liquid states. The gas evolved is a mixture of hydrogen, nitrogen, carbon monoxide, carbon dioxide and methane, and although the bulk of the gas is normally hydrogen it has been necessary to develop an analytical technique to deal with mixtures of these gases. Oxygen is not usually present, but must be looked for. A certain amount of water vapour is also collected, which is desorbed from the oxide skin present on the metal; it cannot be measured accurately in the present apparatus without fairly elaborate precautions and its determination will not be discussed here. The apparatus is similar to one previously described,[12] but incorporates certain important modifications which ensure greater accuracy and, in particular, enable methane to be determined. This was not possible with the earlier apparatus.

PRINCIPLES OF THE ANALYTICAL METHOD

General Consideration—There is a tendency, in many of the methods that have been proposed for micro-gas analysis at low pressures, to make use of certain chemical reagents in a fairly orthodox manner. For example, in the method described by Prescott and Morrison copper oxide is used for the oxidation of hydrogen and carbon monoxide, magnesium perchlorate for the absorption of water vapour, and soda-lime to remove carbon dioxide. These reagents are used in the form of small plugs containing only a fraction of a gram of powder through which the sample gases are circulated by means of automatic Toepler or miniature diffusion pumps. It is necessary, of course, to bake out each of the reagent tubes before beginning an analysis.

We have not used this type of procedure, and have endeavoured instead to make the fullest possible use of physical methods of separation, so that chemical reaction is limited to simple combustion, if possible at a neutral surface, with added oxygen. The use of powdered reagents in a vacuum system we consider undesirable for the following reasons.

(1) The relatively large specific surface of the reagents may result in general absorption of the constituents of the sample.

(2) The necessity for circulation of the gases by Toepler pumps complicates the apparatus and increases the possibility of contamination of the sample.

(3) The absorption by reagents, such as soda lime, is irreversible for practical purposes, so that re-measurement, which is so convenient a feature of the physical methods, is not possible.

The most desirable procedure would be one which gave complete separation of the sample into the constituent gases, so that they could then be measured individually. This ideal can nearly be achieved, since condensable gases and hydrogen are separated by the procedure to be described, and nitrogen is left as a residue; the oxidation products from the combustion of carbon monoxide and methane are also collected separately, and can be measured subsequently. Some of the principles involved in the procedure for individual gases are given below:—

Condensable gases—Condensable gases, *i.e.*, those that have a vapour pressure of less than 10^{-5} mm. at the temperature of liquid oxygen (— 183° C.), can in many instances be determined very rapidly by immersing the tip of a small side-tube on the system in a suitable refrigerant and noting the fall in pressure. For example, freezing acetone (— 95° C.) will condense out water but not carbon dioxide, and liquid oxygen will condense out carbon dioxide but not the permanent gases. Since water vapour cannot be measured accurately because of strong adsorption effects it is usually convenient to keep acetone on throughout the analysis and substitute liquid oxygen whenever carbon dioxide is to be measured or removed.

These are the only two condensable gases present in the mixtures we are considering here; if others are thought to be present, it may be necessary to resort to the method of low temperature distillation, which can be readily carried out without any modification to the apparatus. The procedure has been described elsewhere.[12]

Hydrogen—The specific and rapid diffusion of hydrogen through palladium provides a very simple method for the determination of hydrogen, which is to be preferred to the more general procedure of oxidation, because of the avidity with which water vapour is adsorbed by glass. The possibility, however, of interaction at the palladium surface, in addition to the outward diffusion of hydrogen, must not be ignored. Two reactions are known to occur at ordinary pressures, and temperatures above 300° C.[16]

$$2\,CO \rightleftharpoons C + CO_2 \qquad .. \quad .. \quad .. \quad .. \quad (1)$$

$$CO + 3H_2 \rightleftharpoons CH_4 + H_2O \qquad .. \quad .. \quad .. \quad .. \quad (2)$$

These reactions do not appear to take place to any appreciable extent at low pressures (*i.e.*, less than 0·1 mm.). Since liquid oxygen is usually kept on one of the freezing traps when the palladium tube is used, both reactions would be encouraged to go to the right, but tests have shown that both carbon monoxide and methane are in fact completely stable in presence of hydrogen, even when temperatures of 700° to 800° C. are used. In the earlier form of the apparatus only one palladium tube was used, and diffusion of the hydrogen into the air was effected in about five minutes by heating to approximately 700° C.

Test analyses with known mixtures prepared from coal gas have indicated that some breakdown of carbon monoxide in accordance with equation (1) can take place when unsaturated hydrocarbons are also present, presumably because the reaction is promoted by carbon deposited when the hydrocarbons are cracked. Ethylene, which is not completely condensed out by liquid oxygen, is a possible source of trouble in this respect. To guard against the possibility of error from this cause, a palladium filament can be added to the system, and the stability of the sample tested by running this for a few seconds at about 700° C. prior to switching on the palladium tube. This test will also indicate whether oxygen is present in the gas or not.

A palladium tube can be used with complete confidence, if in addition to measuring the drop in pressure in the system a direct measurement is made subsequently of the hydrogen separated by diffusion. This can be done quite readily, and the new apparatus has been modified to make it possible.

An interesting point that may be mentioned here is that when free oxygen is present in the sample, hydrogen can be admitted to the system by momentarily heating the external palladium tube with a reducing gas flame, without any appreciable reaction taking place at the hot surface of the tube. Advantage can be taken of this fact when it is desired to introduce hydrogen for the purpose of determining oxygen by reaction at the platinum filament.

Carbon Monoxide and Methane—The residual gases after removal of water vapour, carbon dioxide and hydrogen are carbon monoxide, methane and nitrogen. All the methods available for the separation of carbon monoxide and methane at low pressures involve differential oxidation.

The procedure for carbon monoxide and methane together described by Prescott and Morrison is the only one that has been published so far. In their method the carbon monoxide is oxidised by two or three passages over powdered copper oxide heated to 300° C., and the carbon dioxide formed absorbed by soda-lime. The residual gas is then mixed with 2½ to 3 times its volume of oxygen and then compressed by a Toepler pump into a small explosion pipette and sparked at a pressure of about 17 cm. The oxidation products are determined by absorption in magnesium perchlorate and soda-lime.

Since we wished to avoid this type of procedure we have investigated the conditions necessary for the determination of carbon monoxide and methane by simple combustion with oxygen at a platinum filament. If methane is known to be absent the procedure is quite straightforward. The reaction of carbon monoxide with a slight excess of oxygen is very rapid, *e.g.*, with the filament at 700° C.,* and with liquid oxygen on one of the freezing traps to condense out the carbon dioxide formed, the oxidation is complete in a few seconds.

The oxidation of methane is very slow at 700° C. and is incomplete after one hour. If the filament temperature is reduced to about 500° C., the oxidation of carbon monoxide is still so rapid that reaction is complete within two minutes, whereas the methane reacts only to a negligible extent in this time. This procedure is therefore suitable for the first step in this stage of the analysis, *i.e.*, the separation of the carbon monoxide.

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* The filament temperatures cited are true temperatures obtained by applying the appropriate brightness correction to temperatures observed with a disappearing-filament pyrometer.

In order to obtain a sufficiently rapid oxidation of methane, it is necessary to raise the filament to somewhere in the neighbourhood of 1000° to 1200° C., but at this temperature a complication arises in the form of a reaction between the platinum and oxygen. This has been remarked upon by Prescott and Morrison, who state that "contrary to expectation, (the filament) is readily attacked by oxygen above 700° C., to an extent sufficient to limit its use to the combustion of oxygen with an excess of hydrogen or carbon monoxide." We have not been able to find any other references in the literature to this behaviour of platinum. We have, however, carried out a certain number of tests which indicate that:—

(1) When a carefully decarburised platinum filament is heated to temperatures around 1000° to 1200° C. in a low pressure of oxygen there is a rapid and complete disappearance of the gas. For example, at 1150° C. 7·8 μ. of oxygen in the analytical system (volume 540 ml.) cleaned up completely in 10 minutes. The rate of reaction is quite negligible at 700° C., but increases rapidly with rising temperature.

(2) The normal thermal evaporation of platinum atoms from the filament is much too small to account for the clean-up of oxygen by a gettering action. The bulb containing the filament does, however, show a slight blackening after several runs.

(3) The oxygen can be recovered slowly by subsequently baking out the bulb at 350° C.

(4) The cleaned-up oxygen is quantitatively reduced by introducing hydrogen into the system and reheating the filament.

These results are consistent with the theory that the disappearance is due to formation of a volatile platinum oxide which deposits on the walls of the bulb, where it is fairly easily decomposed or reduced. The precise mechanism has not, however, been elucidated.

If a sample of gas containing only methane and nitrogen is mixed with rather more than double its volume of oxygen and allowed to react at a platinum filament heated to about 1150° C., the pressure drops rapidly, until after five minutes it has reached a constant final value. The methane is completely oxidised, as may be checked by measurement of the carbon dioxide formed, which can be quantitatively recovered from a side tube in which it has been condensed out during the reaction. The excess of oxygen is cleaned up by the filament, and the residual gas is thus nitrogen.

This method (oxidation at a platinum filament first at 500° then at 1150° C.) can be used quite successfully for the analysis of mixtures containing carbon monoxide, methane and nitrogen.

Although sufficiently accurate results are obtained in this way and no cumulative effects can be detected after repeated tests, the rather obscure nature of the oxygen clean-up makes the procedure possibly undesirable. An alternative method for the determination of methane was therefore sought. Some preliminary tests indicated that by using an alumina-coated platinum filament, prepared in a manner which will be described later, a high rate of oxidation of methane could be achieved, whilst the protective layer on the platinum reduced the disappearance of oxygen to negligible proportions. With this filament at 1150° C., oxidation is complete in 3 to 4 minutes, and the amount of oxygen used in the reaction can be determined accurately if desired, by measuring the residual oxygen in the usual way.

APPARATUS

A diagram of the apparatus that we have constructed in accordance with the principles discussed in the preceding section is given in Fig. 1. It contains a two-stage mercury diffusion pump (pump A) which collects the sample and delivers it into an analytical system. The latter can be evacuated by a second, three-stage pump (pump B) which is located below the table level and is backed by a general laboratory vacuum line at a pressure of 2 to 5 mm. of mercury. High vacuum technique is followed throughout, and it should be emphasised that successful results can only be obtained when the whole system is so constructed that a final pressure of about 10^{-6} mm. can be rapidly attained, and then held when the system is isolated from the pump. Except for certain special applications, greased taps are eliminated in favour of mercury cut-offs; four, marked a to d in Fig. 1, are used in the present apparatus.

All measurements are made at constant volume, and the pressure changes resulting from a series of operations are read on both a Pirani gauge (P) and a McLeod gauge (M). The McLeod gauge and the cut-offs are constructed so that changes in atmospheric pressure do not appreciably alter the mercury levels in the system. It will be noticed that the volume of the system in which the sample is first collected is dependent upon the action of pump A. With certain types of jet, the volume defined by a pump in this way can vary quite markedly with the pressure of gas collected in the system;[17] the second stage of pump A, however, consists of a short "parallel" jet which tests indicate to be relatively insensitive to backing pressure, so that the volume of the system is constant over the range of pressures normally used.

We find that a total sample pressure of 1 to 30 microns, in an analytical system of 300 to 600 ml., is usually suitable for an analysis.

It has been demonstrated that no reaction occurs between the gases present in a normal mixture, during transmission through the collection pump.

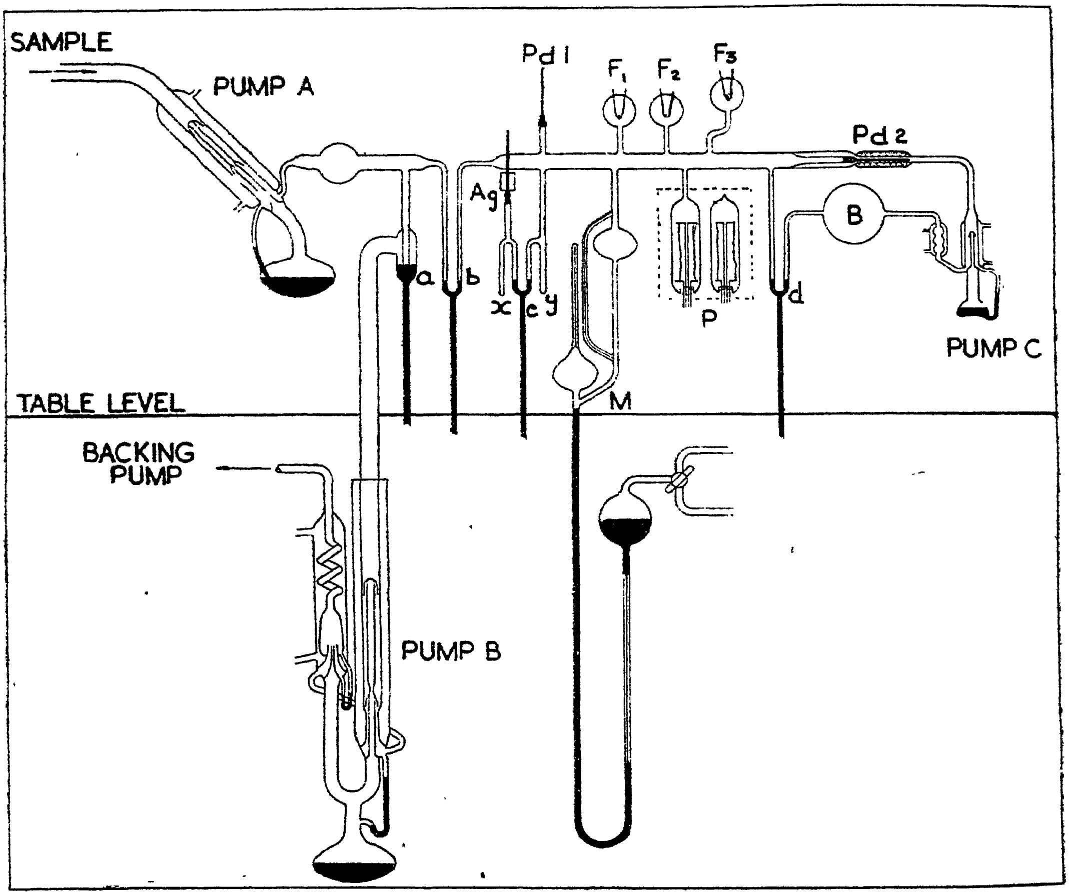
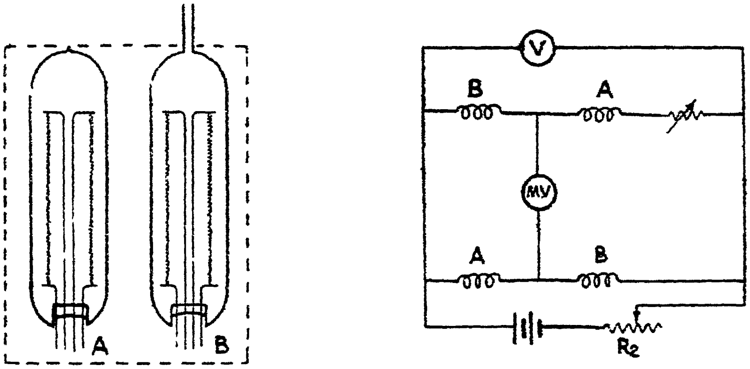


Fig. 1. Diagram of apparatus.

The final pressure measurements at each stage of the analysis are taken on the McLeod gauge, which is compensated for atmospheric variations and requires no adjustment. The single gauge on the present apparatus reads up to about 0·07 mm. of mercury, but for certain experiments it is convenient to use a double gauge with an additional "coarse" bulb capable of reading up to 1 or 2 mm. Such an arrangement is very useful when carrying out determinations of minor constituents. The Pirani gauge is extremely useful for taking continuous readings, and is normally operated in addition to the McLeod. It consists of two identical bulbs, each containing two coiled tungsten filaments of as nearly as possible the same resistance. One bulb is highly evacuated and sealed off, and the other is connected to the system. The method of using this gauge is shown in Fig. 2; the filaments are arranged as a bridge circuit, and a small variable resistance enables any zero adjustment to be made and the bridge balanced. A constant voltage is maintained across the bridge, and the out-of-balance voltage resulting from the pressure of gas in bulb B is measured on a high resistance (2000 ohm) millivoltmeter. This is a very convenient way of operating the gauge, since the continual adjustment required in the more usual null method is avoided. Typical calibration curves for hydrogen and oxygen are given in Fig. 3, and show that the response is linear over the range of pressures normally used. The response is also additive and, since the common gases give calibration curves very close to that of oxygen, the gauge readings can provide useful information during the analysis, particularly in operations involving addition or subtraction of hydrogen. Also condensable gases, such as water, sulphur dioxide and ammonia, which cannot be measured on a McLeod gauge, can be measured by means of the Pirani gauge.



Pirani Gauge

Electrical arrangement for deflection method

Fig. 2.

Two palladium tubes (Pd 1 and Pd 2 in Fig. 1) are incorporated in the present apparatus. They are both of 1 mm. inside diameter and 0·15 mm. wall thickness, with an over-all length of 50 mm. One end is closed by welding and the other is soldered with gold into a platinum thimble, which is in turn joined to the glass of the system.

The tube Pd 1 can be used for admission of hydrogen to, or its extraction from, the analytical system. Hydrogen is admitted by flicking the tube with a soft blowpipe flame, or diffused out by heating it with a miniature electric furnace.

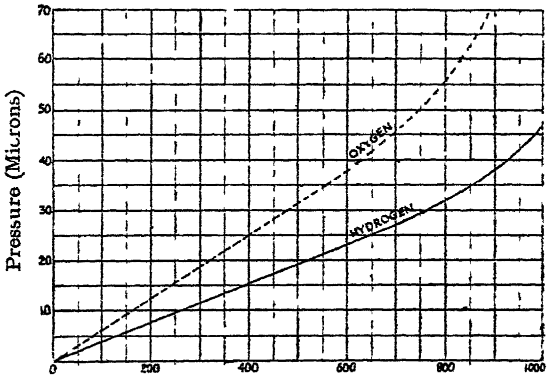


Fig. 3.

Pd 2 is arranged so that hydrogen extracted through this tube can be collected and measured separately. It is therefore sealed internally into the analytical line and protrudes into an extension of the line which is wound externally with nichrome wire and insulated with asbestos cement. This section of tubing is made of a special glass that will withstand 700° C. under vacuum. The temperature attained by the palladium tube is previously calibrated (under vacuum conditions) against the current passed through the furnace winding. It is normally operated at 450° C. and at this temperature the extraction of hydrogen is complete in 15 to 20 minutes. As will be seen from Fig. 1 the outside of Pd 2 is pumped continuously by a very small but fast single-stage mercury pump (pump C); this delivers any hydrogen diffusing through the tube into the bulb B which is evacuated before starting an analysis and then isolated from the main system by means of cut-off d.

The tube Ag in Fig. 1, is used to admit pure oxygen into the system. It is a fine silver tube 2 mm. in diameter, 0·2 mm. in wall-thickness and about 100 mm. long, closed at one end and silver-soldered into a platinum thimble. A radiation fin is clamped on the lower end to prevent overheating of the thimble and the joint to the glass. By heating the end of the tube with a miniature electric furnace running at about 750° C. a controlled volume of oxygen can be allowed to diffuse in from the air.

The three filaments used (F_1, F_2 and F_3) are housed in separate lead-glass bulbs of about 30 ml. capacity. The lead-in wires in each bulb are of 1-mm. diameter platinum. F_1 is a 3-cm. loop of very thin palladium strip, and F_2 a similar length of 0·1-mm. diameter platinum wire. F_3 is the platinum filament coated with alumina. It is prepared by repeatedly dipping a short spiral (0·1-mm. wire coiled on a 0·5-mm. mandrel) into a dilute dispersion of α-alumina in Nicoloidine, with intermediate firing to about 1600° C. in a gas flame; in this way a dense and coherent layer is sintered on to the wire. The bulb containing this filament is off-set from the line of the system to localise any clean-up effects.

The small glass side tubes x and y are used for freezing out condensable gases. Any gas condensed out in x can be isolated from the main system by raising cut-off c. The latter can also be used if necessary to isolate the silver tube during the admission of oxygen.

PROCEDURE

If the analytical system has been open to the atmosphere for any length of time it is advisable to flash the filaments and heat the palladium and silver tubes prior to starting an analysis. The platinum and palladium filaments are heated to 800° C., and the platinum-alumina filament to 1200° C. for 1 or 2 minutes in vacuum. The internal palladium tube should also be "flushed" by allowing some hydrogen to diffuse through it at the normal operating temperature. After this hydrogen has been pumped away, cut-off d is raised to shut off the bulb B from the system.

The sample is introduced *via* pump A, and the pressure in the analytical system, if greater than required, is reduced to 10 to 20μ by manipulation of cut-offs a and b. The sample is finally confined by cut-off b.

Pressure readings are then taken after each of the following steps—

(1) Apply freezing acetone and then liquid oxygen to side-tube x, and raise cut-off c.
(2) Run the palladium filament (F_1) at 700° C. for a few seconds. If the sample is not stable, run the filament for as long as a pressure change occurs and then re-trace the the operations of step (1).
(3) Heat the internal palladium tube Pd 2 at 450° C. for 15 to 20 minutes.
(4) Introduce oxygen equivalent to rather more than half the total pressure, by heating the silver tube (with cut-off c raised during the diffusion).
(5) Heat the platinum filament (F_2) at a current giving an extrapolated temperature of 500° C., for two minutes, with liquid oxygen on y.
(6) Determine the carbon dioxide produced in step (5) by replacing liquid oxygen by freezing acetone.
(7) Determine the residual oxygen by admitting excess of hydrogen through Pd 1, re-heating the filament and finally heating Pd 1 with a miniature furnace at 700° C.
(8) Introduce further oxygen, equivalent to rather more than double the residual sample pressure. Run the platinum-alumina filament at 1150° C. for 3 to 4 minutes with liquid oxygen on y again.
(9) Determine the carbon dioxide produced in step (8).
(10) Determine the residual oxygen by the procedure in step (7).
(11) Pump away the residual gas (nitrogen) and re-determine the carbon dioxide produced in the oxidation steps, and originally present in the sample.
(12) Determine the hydrogen extracted originally, by lowering cut-off d, thus expanding the gas collected in bulb B into the analytical system.

This procedure is more complete than is usually necessary, and enables the carbon monoxide and methane contents to be cross-checked in three ways. For normal rapid analysis it is simpler to introduce initially sufficient oxygen to consume both gases, and then calculate their respective percentages from the fall in pressure when the two filaments are burned.

Analytical results

The accuracy of the method has been tested for the single pure gases, and for binary synthetic mixtures prepared in the analytical system. For more complex mixtures we have followed the practice of carrying out low-pressure analysis of samples taken simultaneously with large samples suitable for orthodox chemical analysis (*e.g.*, Orsat). The normal accuracy is such that constituents are determined with an approximate maximum error of 2 per cent. on the basis of the total sample. This was illustrated in the earlier paper[12] for mixtures containing carbon dioxide, carbon monoxide, hydrogen and nitrogen.

To test the accuracy of the procedure for mixtures containing methane, a sample of coal gas, freed from carbon dioxide and unsaturated hydrocarbons, was collected in a bulb suitably equipped for introducing very small doses of gas into the analytical system. Another sample, taken at the same time, was analysed on an Orsat apparatus. The results obtained by macro-analysis and low-pressure analysis are given in Table I.

Table I

Analysis of treated coal gas sample

Volume of system 540 ml.

		Composition per cent			
Method	Volume taken, ml.	H_2	CO	CH_4	N_2
Orsat	100	44·3	13·9	21·3	Rest
Low pressure	0·0050	44·0	14·3	22·6 (*a*)	,,
	0·0091	46·9	15·2	19·7 (*a*)	,,
	0·0118	45·0	12·0	23·1 (*a*)	,,
	0.0122	44·8	12·6	22·4 (*b*)	,,
	0·0113	46·0	12·1	21·7 (*b*)	,,

(*a*) Using bare platinum filament.
(*b*) Using platinum - alumina filament.

The accuracy illustrated is ample for work on gas extraction from metals, and is, in fact, quite adequate for most micro-analytical purposes.

Minor constituents

In certain circumstances a high degree of accuracy can be obtained in the determination of minor constituents by low-pressure methods; this is specially so when a large sample (*e.g.*, about 1 ml. or so) can be taken initially to give in the analytical system a pressure of about 1 mm. which is measured on a "coarse" McLeod gauge. If the constituent can now be extracted or retained either in its original state or after oxidation or reduction, while the main gases are pumped away, it may then be measured very accurately on the fine McLeod gauge.

We have used this type of procedure to measure the carbon dioxide and carbon monoxide contents of samples of air taken near a large gas-fired furnace. In this instance the carbon dioxide is measured very simply by freezing it out in one of the side tubes, pumping away the permanent gases, and then measuring the increase in pressure when freezing acetone is substituted for the liquid oxygen. For the determination of carbon monoxide a similar sample is taken and the carbon dioxide frozen out and isolated in a side-tube by means of a cut-off; the carbon monoxide is then oxidised by running a platinum filament for a few minutes, and the carbon dioxide produced is measured in the same way as before. Prolonged pumping of the condensate must be avoided, as the vapour pressure of carbon dioxide at — 183° C. is not sufficiently low to be ignored.

The low-pressure method for carbon determinations is based on this type of manipulation, and has a much greater sensitivity than the normal chemical methods. For example, an accuracy within ± 10 per cent. can be obtained on samples containing only 0·001 per cent. of carbon.[18]

When the minor constituent to be measured is hydrogen, it is very conveniently extracted by means of the internal palladium tube, and then measured after the other gases have been pumped away. Table II gives typical results obtained on some mixtures of hydrogen with inert gas which were analysed in this way.

A brief review is given of the development of micro-gas analysis at low pressures, and a new procedure is described for the analysis of mixtures containing carbon dioxide, carbon

TABLE II

ANALYSIS OF HYDROGEN – INERT GAS MIXTURES

Sample	Percentage of hydrogen			
a	1·00	1·02	1·00	
b	3·30	3·25	3·30	
c	4·16	4·20	4·05	4.22

SUMMARY

monoxide, hydrogen, methane and nitrogen. The apparatus is particularly suitable for the collection and analysis of the gases evolved from heated metals, and similar applications. When the volume of the sample is of the order of 0·005 ml., the accuracy of analysis is such that constituents are determined to within ± 2 per cent. of the total sample.

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DISCUSSION

Mr. A. F. **WILLIAMS** asked whether a mixture of condensable gases could be separated into the constituent gases. How would a mixture of carbon dioxide, sulphur dioxide and hydrogen sulphide be separated.

Dr. **RANSLEY**, in reply, said that if the condensation temperatures of the constituent gases of a mixture are sufficiently widely separated, so that on warming the condensate the first gas attains its full partial pressure before the temperature rises to the condensation temperature of the second, and so on, the pressure-temperature curve of the mixture will show a distinct plateau between one gas and the next. When this is so, separation of the gases can be achieved fairly readily. A cut-off can be used to isolate the small side tube in which the gases are condensed, and by raising the mercury in this when the condensate has reached a suitable temperature, the most volatile constituent, which has completely evaporated to fill the main system, can be separated from the remaining gases still retained as solids in the side tube.

This procedure was illustrated by reference to slides showing typical vapour pressure - temperature curves of $CO_2 - SO_2$ and $CO_2 - NH_3$ mixtures. The method will not work if the gases form solid solutions when frozen out.

In some instances a freezing agent can be selected to effect a direct separation of two gases; for example freezing acetone (−95° C.) is used to differentiate between water vapour and carbon dioxide, the difference between the condensation temperatures of which is about 90° C.; but where the difference is not so great it may be difficult to find the precise freezing agent for separation.

Regarding the separation of hydrogen sulphide, carbon dioxide and sulphur dioxide, the last two can be separated successfully, but the vapour pressure - temperature curve of hydrogen sulphide is very similar to that of carbon dioxide, and low-temperature distillation cannot be used to separate them. Wooten (*A.S.T.M. Bulletin*, 1941, No. 108, 39) describes a test to distinguish between these two gases, which involves passing the gas over silver oxide at 100° C.; hydrogen sulphide reacts to form water vapour and silver sulphide, but carbon dioxide does not react at this temperature.

Dr. P. L. **ROBINSON** queried whether a true separation of two gases could be effected in this way. When dealing with a mixture of carbon dioxide and water, for example, would not the carbon dioxide be adsorbed on the separated ice ?

Dr. Ransley replied that the effect suggested by Dr. Robinson was not sufficiently large to interfere with a practical separation. The side tube in which the gases are condensed has a very small area compared with the remainder of the wall of the analytical system.

Mr. F. Popper enquired if the micro-fractionation technique had been used for the analysis of hydrocarbon mixtures.

Dr. Ransley said that the method had been applied successfully in this field, *e.g.*, by Sebastian and Howard (*Ind. Eng. Chem., Anal. Ed.*, 1934, 6, 172).

Mr. H. N. Wilson asked whether water vapour adsorbed in the oxide film on aluminium would react with the metal on heating and cause fictitiously high values for dissolved hydrogen.

Dr. Ransley's reply was that there was a real danger that misleading results might be obtained for the true hydrogen content of aluminium if this important factor was not taken into account. The very high values obtained by earlier workers were due to the fact that thin sheet with a large surface area was frequently used, and the surface was not usually prepared in any way. The evolution of some surface gas, originating from this reaction with adsorbed water, is inevitable, but by the adoption of a standard method of preparing samples an appropriate and consistent correction could be made for this spurious gas and the true hydrogen content calculated. He used cylindrical specimens of approximately 1 cm. diameter and 4 or 5 cm. long, which are turned dry on a lathe, washed in benzene, and introduced into the vacuum system as rapidly as possible. With specimens prepared in this way, the correction on a normal gas content is not more than about 10 per cent.

The Analysis of Hydrocarbon Gases by Low Temperature Distillation

By J. H. D. HOOPER

Introduction

The chemist concerned with the quantitative analysis of gases likely to be encountered in the petroleum industry is faced with a highly specialised problem which is moreover complicated by the wide diversity of gaseous mixtures that he may be called upon to examine. Such gases will vary from simple binary systems, for example mixtures of *iso*butane and *n*-butane, to complex paraffin-olefin mixtures containing perhaps a dozen or even twenty constituents Five typical gas mixtures are shown in Table I.

Table I

Composition of typical hydrocarbon gases

Expressed in molecular percentages

	Refinery Gases.			Natural Gases.	
Constituent	A	B	C	D	E
Hydrogen ..	8·6	89·9	—	—	—
Methane	13·5	3·9	—	82·3	42·2
Ethylene	4·3	—	—	—	—
Ethane	8·3	3·3	—	5·3	12·7
Propylene	23·6	—	—	—	—
Propane	5·3	1·3	2·0	1·6	5·4
*Iso*butane	7·8	0·6	70·7	0·2	0·2
*Iso*butene	9·7	—	—	—	—
n-Butenes	15·7	—	—	—	—
n-Butane	3·2	1·0	26·8	0·4	0·5
Pentanes	Trace	—	0·5	0·4	0·3
Carbon Dioxide ..	—	—	—	Nil	0·2
Oxygen	—	—	—	Nil	0·4
Nitrogen	—	—	—	9·8	38·1

For simple gas mixtures, it is often possible to effect an analysis by straightforward chemical means, for example, by absorption of specific components such as olefinic gases in suitable liquid reagents; alternatively, combustible gases may be oxidised by passage over heated copper oxide or a platinum spiral, or by explosion after admixture with oxygen. Analytical techniques employing such procedures are well established and form the basis of the well-known Orsat, Bone and Wheeler, G.L.C. (Gooderham) and Haldane types of apparatus, and many others.

Certain gas mixtures lend themselves to analysis by direct physical methods, *e.g.*, by by measurement of dew pressure, vapour pressure or thermal conductivity. Recently, spectroscopic methods involving the measurement of absorption of infra-red or ultra-violet radiation have proved valuable tools in the hands of the gas analyst. In America, much progress has also been made in the development of the Mass Spectrograph for the analysis of gases. Nevertheless, with the possible exception of the Mass Spectrograph, the methods indicated here cannot give a complete analysis of a complex multi-component hydrocarbon gas mixture such as sample A in Table I. They can, however, be employed with advantage when such a mixture has been split up into a number of simple fractions, each containing relatively few constituents. If the boiling points of the lighter hydrocarbons are considered, (Table II) it will be seen that the limited number of possible hydrocarbons, containing between one and five carbon atoms per molecule, are spaced over a boiling-point range of nearly 200 degrees Centigrade: this fact therefore, provides an obvious basis for the physical and analytical separation of the hydrocarbon gases.

TABLE II

BOILING POINTS OF SOME GASEOUS HYDROCARBONS

Hydrocarbon	Boiling Point, °C.
Methane	− 161·4
Ethylene	− 103·9
Ethane	− 88·9
Propylene	− 47·7
Propane	− 42·2
*Iso*butane	− 11·8
*Iso*butene	− 6·8
Butene-1	− 6·3
Butadiene	− 4·5
n-Butane	− 0·5
Butene-2 (Trans.)	+ 0·9
Butene-2 (Cis.)	+ 3·65
*Iso*pentane	+ 27·95
n-Pentane	+ 35·95

During the past 15 to 20 years much work has been carried out along these lines and numerous pieces of equipment for the fractional distillation of condensed gases have been described in the literature. The best known apparatus is undoubtedly that due to Podbielniak,[1,2,3] and several versions of his apparatus are available commercially.

GENERAL DESCRIPTION OF APPARATUS

Basically, an apparatus for the low-temperature distillation of gases comprises the following main features.

(*a*) A distilling bulb, generally of capacity from 10 to 100 ml., and fitted with an electric heater.

(*b*) A glass column of small bore (3 to 5 mm.), containing a wire packing.

(*c*) A sleeve-type vacuum jacket enclosing the column and either internally silvered or provided with an internal metal reflector.

(*d*) A condenser at the column head for providing liquid reflux, liquid air or preferably liquid nitrogen being used as a refrigerant.

(*e*) A stopcock manifold, for the transfer of gaseous distillate to the receivers, to which are connected manometers for measurement of column and receiver pressures.

(*f*) Distillate receivers. Two forms are commonly used—

  (i) Large graduated burettes in which the distillate is collected (and directly measured) by displacement of a suitable confining agent such as mercury or brine.

  (ii) Vacuum bottles, normally of capacity between 4 and 15 litres, maintained in a water-bath at constant temperature. This type of receiver, in which the quantity of distillate collected is assessed by measurement of rise in pressure, is employed in the apparatus described in this paper.

(*g*) Miscellaneous items such as a scrubbing train to remove carbon dioxide and water vapour from gases prior to entry into the distilling bulb, a vacuum pump and a Töpler pump to enable gas samples to be withdrawn from the receivers. A thermocouple is provided for measurement of column-head temperature.

A simplified diagram of an apparatus incorporating these features is shown in Fig. 1.

OUTLINE OF DISTILLATION PROCEDURE

Although many details of distillation procedure[4,5,6] will vary according to the type of gas sample under examination, a general account of the method may be given briefly, as follows.

The apparatus is first evacuated and tested for leaks. If these are absent, the column head and distilling bulb are cooled by means of liquid nitrogen, the degree of cooling depending on the nature and boiling-point range of the sample. The hydrocarbon gas sample, free from moisture and carbon dioxide, is then cautiously admitted to the cooled distilling bulb, where it condenses. If gases such as air or hydrogen are present, however, these will not be condensed, and the pressure in the system, as indicated by the column manometer, will rise rapidly. Such gases are therefore bled off into one of the receivers, while the remainder of the sample is being liquefied.

The quantity of gas charged to the apparatus will depend on the composition of the sample. An average figure would be 2 to 3 litres; if, however, the proportion of "uncondensable" gases is high, as in example B, Table I, four or five times this amount of gas would have to be passed into the apparatus to allow an adequate liquid charge to accumulate in the distilling bulb. It is in fact, normally desirable to collect at least 10 ml. of liquid in this bulb.

When sufficient gas has been condensed, the sample inlet tap is closed and the receiver manifold is isolated from the column. Excess of refrigerant is removed from the Dewar vessel enclosing the distilling bulb, and current to the heater windings is switched on. The pressure in the column tends to rise as the charge becomes warm, and is restored to atmospheric by taking off uncondensable gases into the receiver, or by cooling the column head if liquid reflux has appeared on the packing. When the column is in equilibrium under conditions of total reflux and the temperature indicator is recording the boiling point of the lightest hydrocarbon present in the mixture, take-off of distillate is begun. The overhead temperature and the receiver pressure are recorded at frequent intervals to allow an accurate distillation curve to be plotted. The rate of removal of distillate will again depend on the nature of the sample; where the distillation curve shows a long "plateau," due to the presence of a large proportion of a single component, relatively high take-off rates, say, from 20 to 50 ml. per minute, may safely be employed. At "breaks" between two components. and particularly where these have boiling points that are not widely separated, it is necessary, for good fractionation, to operate at a high reflux ratio and hence a very low take-off rate, possibly only 2 to 5 ml. or less, per minute.

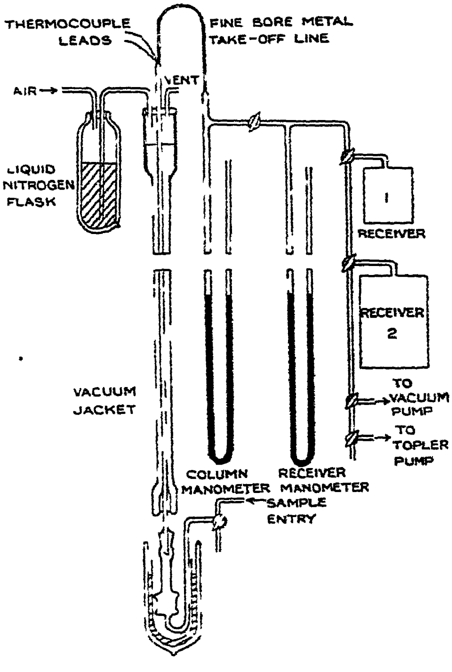

Fig. 1 General layout of simple low-temperature distillation apparatus.

For hydrocarbons in the methane – butane boiling-point range, it is usual to maintain the column at atmospheric pressure, but for higher-boiling materials it is necessary to reduce the pressure in the column to a value sufficiently low to ensure that liquid hydrocarbon will not tend to condense in the manifold. Thus a convenient distillation pressure for pentanes is 300 mm. and for hexanes 100 mm. of mercury (absolute). In addition, pressure in the distillate receiver must not be allowed to rise above certain limiting values (approx. 300 mm. Hg. for C_1 to C_4 hydrocarbons, and correspondingly lower for pentanes and hexanes), otherwise errors due to the deviations of the various hydrocarbons from the ideal gas laws become significant. If such errors can be neglected, the calculation of results is greatly simplified, as it can be assumed that the rise in receiver pressure for each fraction is proportional to the volume of that fraction present in the total gas sample. The latter quantity is deduced from the total recorded pressure rise, after due allowance has been made for difference in size between the receivers used. It is then a simple matter to calculate the volume percentage of each fraction present. If the fractions contain constituents that cannot be quantitatively estimated from the distillation curve, for example, gases such as nitrogen, hydrogen, or unsaturated compounds, it will be necessary to withdraw samples of these fractions from their individual receivers for further analysis by absorption, combustion or spectroscopic methods. It will be seen from the typical distillation curve shown in Fig. 2, that it is convenient to divide the gas into groups of hydrocarbon fractions containing 1, 2, 3, 4 and 5 carbon atoms per molecule respectively. If we consider the gas denoted by A in Table I, the fractions taken will contain the following constituents:—

Fraction 1—Hydrogen, methane (and uncondensibles, if present).
Fraction 2—Ethylene, ethane.
Fraction 3—Propylene, propane.
Fraction 4—*Iso*butene, *n*-butenes, *iso*butane, *n*-butane.
Fraction 5—Pentenes, pentanes.

Analysis of these fractions in subsidiary apparatus thus allows the ultimate composition of the gas sample to be determined.

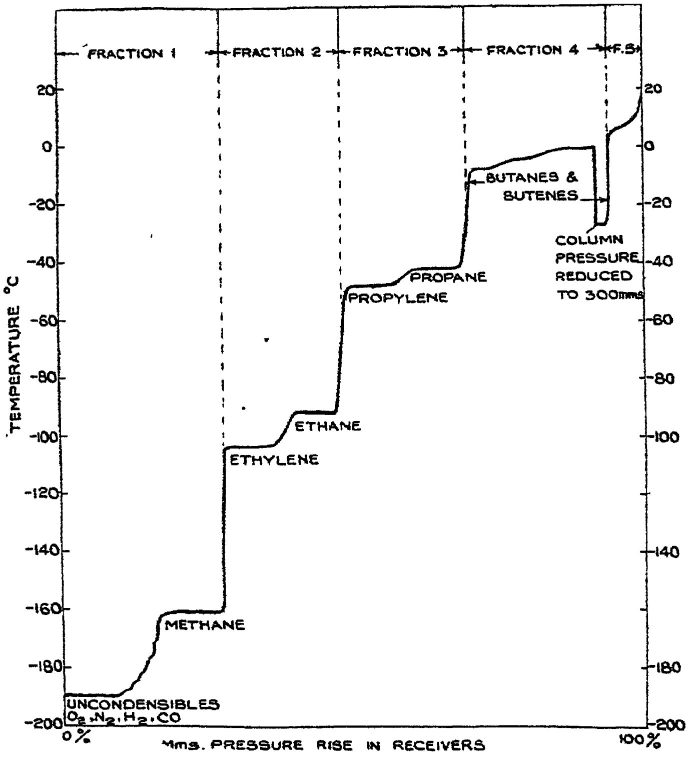

Fig. 2. Example of low-temperature distillation.

TYPICAL LOW-TEMPERATURE DISTILLING COLUMN ASSEMBLIES

Most of the earlier low-temperature distillation columns employed a relatively simple form of packing, often just a single wire helix, because designs of this nature allowed reasonable fractionating power without causing excessive liquid hold-up. In view of the small charge employed, this latter factor is, of course, a matter of some importance. Details of two typical columns are given in Table III.

Numerous types of this form of packing have been suggested from time to time; packings have been constructed from wire of all gauges and from ribbon and have been wound in a variety of pitches. Double helices have been used, and the addition of a straight wire former down the centre of the helix has also been recommended. As an example of the latter, a design by McMillan[7] may be described. This comprised a 6-turn-per-inch 15-gauge brass spiral with a straight central insert of 23-gauge wire. As a matter of interest it might also perhaps be mentioned that the author once experimented with an empty tube, 4 mm. in diameter, containing no packing at all, and achieved reasonable separation of a mixture of *iso*butane and *n*-butane. Columns of the general type so far described were satisfactory for the separation of many hydrocarbon mixtures, but were not adequate for the fractionation of compounds boiling much closer together than 8 degrees Centigrade. The majority were perhaps equivalent to about 7 to 15 theoretical plates.

TABLE III

SIMPLE LOW-TEMPERATURE DISTILLING COLUMNS

Column	(1)	(2)
Type of packing	Single Helix.	Single Helix.
Length of packing, cm. .. .	108·5	99
Internal diameter of column, mm.	3·7	3·0
Gauge of wire (S.W.G.) ..	23	26
Turns per inch	5·7	3·8

Two further packings will be mentioned briefly. First, the low-hold-up twisted gauze design described by Bower and Cooke,[8] when used in a column 5 mm. in internal diameter, has been found very suitable for this type of work; secondly, reference must be made to the Heli-Grid column introduced by Podbielniak.[9] This is of complex construction, and consists of a number of sector-like coils of fine wire, wound around each other and also round a straight central core. This column has, of course, a much higher hold-up than the simple helix, but this is offset by the greatly increased fractionating efficiency. It is, in fact, claimed by Podbielniak to be equivalent to 50 or more theoretical plates. It is certainly the author's experience that it can be used to separate such close-boiling components as *iso*butane (b.p. −11·8° C.) and *iso*butene (b.p. −6·8° C.), or methylethylethylene and *n*-pentane. Typical separations achieved with a Heli-Grid column are shown in Table IV.

TABLE IV

EXAMPLES OF SEPARATIONS OBTAINED WITH "HELI-GRID" TYPE COLUMN

The figures are expressed in molecular percentages

Component	B.p. °C.		Mixtures I	II	III	IV	V	VI
*Iso*pentane	27·95°	% *blended*	—	—	*47·5*	*2·3*	*31·4*	*38·9*
		% found	—	—	47·2	2·85	31·85	38·6
Methylethylethylene ..	31·1°	% *blended*	*13·9*	*6·95*	*52·5*	—	—	—
		% found	14·7	8·05	52·8	—	—	—
n-Pentane	35·95°	% *blended*	*86·1*	*93·05*	—	*97·7*	*68·6*	*61·1*
		% found	85·3	91·95	—	97·15	68·15	61·4

Many improvements have also been made to the design of the vacuum jacket and the condenser unit. It should be appreciated that during a distillation the temperature of the reflux zone will slowly rise from − 190° C. to almost room temperature. It is therefore important to be able to maintain the condenser at any required constant temperature within this range. At the same time the heat capacity of the condenser must not be excessive, otherwise the delays incurred at cut-points, when the reflux zone temperature rises from the boiling point of one component to that of the next highest, will lead to unduly prolonged distillations. The simplest form of condenser is an annular metal tank, concentric with the upper portion of the column,[1] and supplied with refrigerant as necessary. A more elaborate design proposed by McMillan[7] consists of a layer of aluminium pellets which are packed round the column and can be cooled by liquid nitrogen trickling down from a small copper sprinkler tank.

The earlier vacuum jackets were often subject to breakages owing to contraction of the inner wall on cooling to a temperature some 200 degrees below that of the outer wall. This difficulty can be overcome by the use of flexible glass bellows. Another modification has been the enlargement of the lower portion of the jacket to accommodate the whole of the distilling bulb in addition to the column. The Podbielniak Super-Cool type of jacket incorporates both these features.[9,10] The complete jacketing of the distilling bulb eliminates the atmospheric weathering of the charge, often encountered with the older, external bulb, and permits more rigid control of heat input, and hence greater column stability. Another feature of this jacket is the inclusion of a free vapour space between the column and the inner walls of the jacket. This allows liquid nitrogen vapour from either the condenser or the distilling bulb compartment to gain access to the entire length of the column and is of great assistance during the preliminary process of cooling the apparatus, saving both time and refrigerant. Similarly, dry air, warmed if necessary, may be passed up over the column walls during the course of a distillation. This air current tends to overcome the heat capacity lag of the column and reflux cooling unit, particularly at "breaks," and is also of much value towards the conclusion of a distillation when the bulb is empty and it is desired to distil off the residual liquid reflux adhering to the packing.

AUTOMATIC DEVICES

It will be clear that the carrying out of a low-temperature distillation requires considerable skill and attention on the part of the operator. Frequent readings of temperature and receiver pressure must be taken and recorded, heat input and gas take-off rates must be carefully adjusted to suit the fraction being distilled, and column pressure must be maintained at as constant a level as possible to allow a smooth distillation curve to be obtained. Careless operation, or perhaps even only a moment's inattention, may lead to poor fractionation, "flooding" in the column or the "blowing" of manometers. Much thought therefore has been devoted to the use of automatic devices that will relieve the task of the operator. Perhaps the greatest boon in this respect has been the development of means for the automatic control of column pressure. The mode of operation of a typical system is indicated in Figure 3. It is controlled by contact wires dipping into the open limb of the column pressure manometer. A rising mercury level, due to increase in column pressure, completes an electrical circuit and by means of any convenient relay system, opens a solenoid valve on a compressed-air line connected to a liquid nitrogen storage vessel. Refrigerant is thus displaced from this vessel into the reflux cooling unit, and this causes the column pressure to fall again.

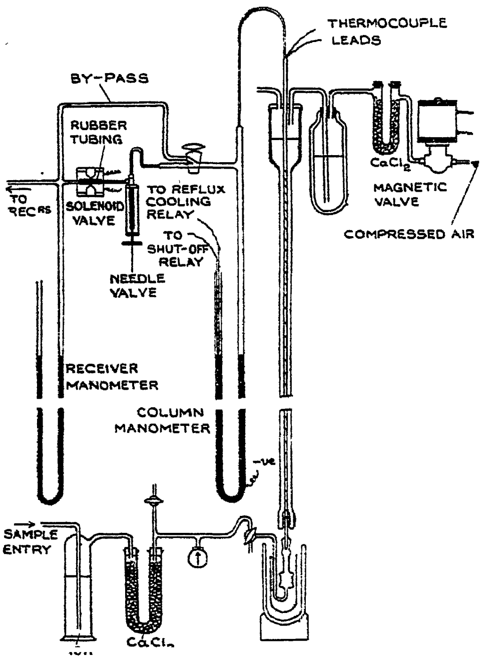

Fig. 3. Semi-automatic low-temperature distillation apparatus.

Similarly, if the column pressure should drop below a predetermined level, the falling mercury in the manometer breaks a circuit, and take-off of gas distillate is stopped by the closing of a very simple shut-off valve, until the pressure builds up once more. This valve consists of a short length of flexible tubing resting between two metal jaws: the lower jaw is fixed, and on operation of the valve the upper jaw is forced downwards by the action of a solenoid, thus gripping the tubing tightly and preventing passage of gas.

A further refinement is the incorporation of an additional contact wire in the manometer, which will cause a buzzer to sound and draw the operator's attention to any unusually high pressure rise, caused, perhaps, by exhaustion of the liquid nitrogen in the storage vessel.

Podbielniak has introduced an almost entirely automatic distillation apparatus,[9,10] which is even capable of plotting its own distillation curve. This is achieved by means of an ingenious device whereby a contact point at the base of a thin movable rod, dipping into the atmospheric limb of the receiver pressure manometer, is automatically raised by a motor-driven rack-and-pinion mechanism each time it is touched by the rising mercury. The motor that operates this mechanism is coupled to the drum carrying the chart on which the column head temperature is plotted by means of a recording potentiometer. The forward movement of the chart is therefore directly proportional to the rise in pressure in the receivers and hence to the quantity of gas distilled. In this apparatus, column pressure is automatically controlled by means similar to those already described and, in addition, take-off rate is automatically varied as the distillation proceeds. On plateaux, distillate will be admitted to the receivers at a reasonably fast rate, but as a cut point is approached the take-off rate is progressively reduced, in order to improve fractionation at the breaks. This rate-control device responds to the smallest tendency towards rise in overhead temperature as detected by the potentiometer galvanometer, and also to falls in column pressure: either effect causes (by means of suitable relays) a solenoid valve to open and inject water into a hydraulically operated needle-valve which reduces the flow of gas through the distillate manifold.

An apparatus of this nature, although elaborate and expensive, does nevertheless relieve the operator of many tasks and, by carrying out such tasks automatically and mechanically, results in greater standardisation of procedure and hence in increased accuracy of analysis. Moreover, with automatic distillation apparatus it is possible for an operator to carry out two or even three distillations simultaneously.

Finally, the author would like to express his thanks to Dr. D. A. Howes and the Directors of the Anglo-Iranian Oil Company for permission to present and publish this paper, and to Mr. C. A. Miller for his assistance in the preparation of much of the material.

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DISCUSSION

Mr. F. POPPER enquired what was the advantage of gas collection with pressure measurement over collection with volume measurement.

Mr. HOOPER replied that both systems had been used in low-temperature distillation apparatus for assessment of gas quantity, and both have their own advantages and disadvantages.

(*a*) For pressure measurement, the gas must be collected in evacuated receivers, and the slightest leak is a major source of error.

(*b*) With volume measurement, there is less risk of leakage, but it is necessary to collect the gas over a confining liquid. For small fractions, mercury can be used for this purpose, but this is obviously impracticable where quantities of the order of a litre or more are involved. It is therefore generally necessary to use brine or some other aqueous confining agent in which the various hydrocarbons are not readily soluble. Even then, it is not convenient to use graduated burettes much above 2 litres in capacity, and this is a disadvantage when large fractions of, say, 10 litres in size have to be collected, unless the apparatus is equipped with a considerable number of receivers. Since each burette requires two taps the use of such equipment means a multiplicity of taps, and consequent additional time must be allowed for their maintenance, *i.e.*, greasing, etc. Control of gas take-off rate can, of course, be readily achieved by control of out-flow of liquid, and gas volume can be measured directly by observations of liquid level. This advantage, however, is offset by the fact that, since volume measurements are made at atmospheric pressure, gas law deviations become significant, particularly for hydrocarbons in the butane range, and it is necessary to apply suitable corrections to observed volume readings; in addition the vapour pressure of the confining solution must also be taken into account. Finally, it is not easy to use such a volume measurement system when operating the column at reduced pressure for the distillation of pentanes and heavier hydrocarbons. It is possible under such conditions to withdraw the confining solutions from the burettes by attachment of these to an evacuated reservoir, but this does not permit ready control of the column pressure at a constant value.

For the above reasons, the speaker was inclined to favour the system of gas collection using pressure measurements.

Mr. R. E. DODD said that, given that it would be possible to use Mr. Hooper's procedure to separate, say, C_2 and C_3 fractions and examine by infra-red spectroscopy, was the separation sufficient to avoid the strong ethylene band at 12 μ, obscuring the C_3 spectrum?

Mr. HOOPER replied that the boiling points of the C_2 and C_3 hydrocarbons are sufficiently widely spaced to permit clear-cut fractionation of such a mixture into two fractions, one containing ethylene and ethane only and the other containing propylene and propane. With careful fractionation, no C_2 component should be present in the C_3 fraction, and *vice versa*, particularly if the Heli-Grid type column was used. If, as a result of unsatisfactory operating technique, a poor "cut" was made, with the result that a small quantity of C_2 hydrocarbon did appear in the C_3 fraction, this would be ethane rather than ethylene, since the latter, which has the lower boiling point, would already have been distilled off before the C_2–C_3 cut point was reached. Thus the risk of ethylene interference in the C_3 spectrum during spectroscopic analysis of the C_3 fraction would appear to be slight.

A New Apparatus for Gas Analysis by the Soap Film Method

BY W. J. GOODERHAM

A NEW method of gas analysis was described[1] and demonstrated[2] by the author in 1940, when it was pointed out that "given a series of accurate gas meters, which do not appreciably absorb gas or cause loss of pressure, the simplest form of gas analysis can be obtained by passing gas through a meter, a reagent to remove completely one constituent of the gas, another meter, another reagent, another meter and so on." The meters used were soap films, whose rates of travel were measured over calibrated volumes with a stop-watch. Since then, a new type of soap film meter has been developed, which enables the complete analysis to be obtained practically directly in percentages without the use of a stop-watch.[3]

NEW METER

One form of the new meter is shown in Fig. 1. Gas enters at A and normally passes into the top of the cup R, which contains Aerosol soap solution,[1,4] and upwards through tube F and the calibrated parts of the meter to the point H and out to the scrubber. By raising tube C or D, it becomes possible to push up the wire E, so that tube F is sealed in the soap solution and gas can no longer pass up the length of the meter. When F is sealed, however, the tube G becomes unsealed and gas flows through G out to the point H and to the scrubber as before. The soap cup, R, thus acts as a two-way valve, which does not stop the flow of gas but deflects it either past the calibrations (cup down) or through the by-pass (cup up).

The mercury seal serves the double purpose of sealing the bottom of the soap cup and sealing the whole tube from air and thus facilitates the removal of the bottom of the apparatus by cutting away the single rubber connection. The soap film meter can then be washed through with water from the top on the rare occasions when this becomes advisable.

Alternative forms of fitting the soap cups have been used, such as those shown in Figures 1 (*a*), 1 (*b*), and 1 (*c*), and another form of the meter has been described for proving gas meters.[4]

The soap film is broken at the top of the meter by a tightly-rolled spill of blotting paper. This absorbs the thin centre of the film and the remainder drains back into the cup. The blotting paper is held by a loose piece of glass tubing, which rests on the top of the standard ground-glass joint. Both soap solution and blotting paper can be introduced by removing the cap and piece of glass tubing, but the cup should be left up when soap solution is being added, to allow for adequate drainage, otherwise a succession of films will appear.

A soap film is made by raising and lowering the cup R, whereupon the gas travelling up F pushes the film along. The travel of the film can be stopped by raising R and so diverting the flow of gas through G.

APPARATUS FOR ANALYSIS OF COAL GAS*

An apparatus for the analysis of coal gas is shown in Fig. 2, which is drawn to scale except that the head, h_1, for operating the reagent flow is normally only a few inches. The gas should be freed from all tar fog, and preferably partially dried over lump calcium chloride. If it is at a pressure greater than 2 in. water gauge, the gas is allowed to flow in at J and to bubble slowly out to air at K, with the three-way tap, L, turned

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* Messrs. Griffin & Tatlock have been granted a licence to make these apparatuses under B.P. 550,323 and B.P. 489,117.

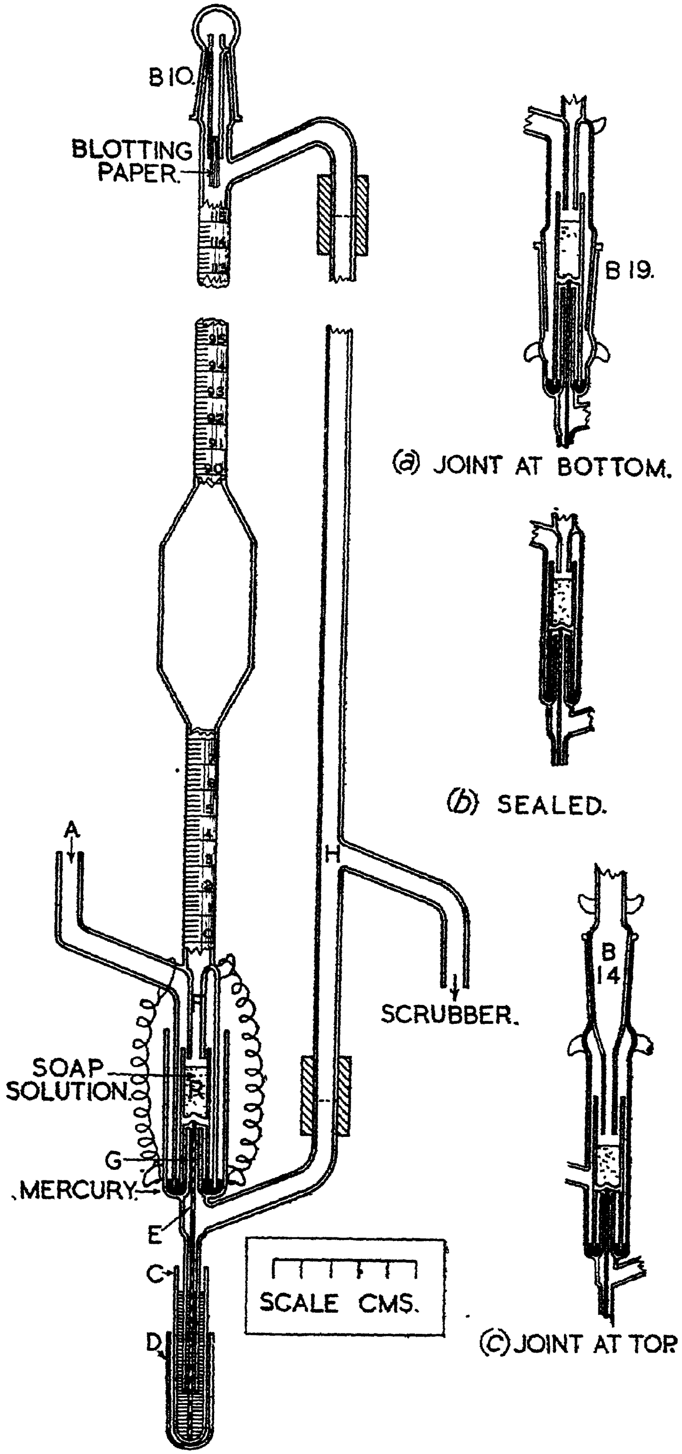
B 10.
BLOTTING PAPER.
A
H
SCRUBBER.
SOAP SOLUTION.
G
MERCURY.
E
C
D
SCALE CMS.
B 19.
(a) JOINT AT BOTTOM.
(b) SEALED.
B 14
(c) JOINT AT TOP.

Fig. 1

as shown in Fig. 2. If the pressure is less than 2 in. water gauge, the clean gas again flows in at J, but passes through the electrically-driven pump with the tap L turned round the other way. The by-pass tap, M, is partially closed in such a way that gas bubbles gently through the blow-off. The blow-off and by-pass should be connected to burners. Whichever way gas is entering the apparatus, part of it, under the small constant head, h, of butyl phthalate, flows through the control capillary at about 20 to 30 ml. per min. to the saturator, which consists of a folded strip of blotting paper dipping into water. Gas, saturated with water vapour, passes through the first soap film meter and then through the first scrubber, which, as described earlier,[1] consists of a glass spiral continuously wetted with 15 per cent. w/w caustic potash solution, having a negligible back pressure. The gas thereafter flows in series through a second soap film meter, a scrubber continuously wetted with activated sulphuric acid (not the activated nitrating mixture described in 1940[1]), a water saturator, soap film meter No. 3, chromous chloride, meter No. 4, copper oxide made from heavy gauge wire and maintained at a temperature of 270° C., meter No. 5, 15 per cent. caustic potash solution, meter No. 6, Arneil catalyst at about 600° C., meter No. 7, 15 per cent. caustic potash solution and finally meter No. 8. Water, formed in the combustion tubes, flows into cylinders, Q, where it can be measured, if desired. The gas, therefore, has the following constituents removed from it in the following order—carbon dioxide, unsaturated hydrocarbons, oxygen, hydrogen, carbon monoxide and paraffin hydrocarbons, until only nitrogen (and other inert gases) are left flowing in the last soap film meter (No. 8).

The reagents, except for 15 per cent. potash solution in lieu of 25 per cent. solution, are the same as those used on the constant volume gas analysis apparatus.[5] The rate of flow of a liquid reagent is about 30 ml. per hour, but is not critical, the flow being controlled in the manner already described.[1].

All the tubes D (Fig. 1) are connected to a single shaft which can be turned to and fro and thus the eight soap cups raised and lowered together. To carry out a gas analysis, gas is turned on to the apparatus with all reagents in operation. Soap films are sent up the meters from time to time until all the glass walls are wetted with soap. This action also flushes out the bottom connections. When all the air has been swept out an analysis can be made. The eight soap cups are raised and lowered and a soap film moves upward in each of the eight meters. When the film in the first meter is at the top of the lower scale, the cups are sharply raised and left in this position. The eight soap films stop and readings can be taken, for example

Meter No. ..	1	2	3	4	5	6	7	8
Reading.. ..	7·4	7·1	6·8	6·4	2·3	0·6	0·5	1·5

The cups are then lowered sharply and flow continues up the main length of the meters until the first film is stopped at 107·4, whereupon the positions of all the other films are read. If the previous figures are subtracted from these readings it becomes possible to obtain the analysis immediately, as can be seen by continuing the example.

Meter No. ..	1	2	3	4	5	6	7	8
Readings: end ..	107·4	104·2	100·9	99·6	48·3	31·8	32·9	10·5
start ..	7·4	7·1	6·8	6·4	2·3	0·6	0·5	1·5
Difference ..	100·0	97·1	94·1	93·2	46·0	31·2	32·4	9·0

Thus, while 100 volumes of the initial gas were passing in meter No. 1, only 97·1 volumes were travelling in meter No. 2, after carbon dioxide had been removed. The percentage of carbon dioxide is thus 2·9 per cent.; similarly, the percentage of unsaturated hydrocarbons is 3·0 per cent., of oxygen 0·9 per cent., of hydrogen 47·2 per cent., and of carbon monoxide 14·8 per cent. The nitrogen concentration is 9·0 per cent. and that of paraffin hydrocarbons (C_nH_{2n+2}), 31·2 — 9·0 or 22·2 per cent. The value of n in (C_nH_{2n+2}) is (32·4 — 9·0)/(31·2 — 9·0) or 1·054. If ethane or higher homologues are present in the paraffin hydrocarbons, combustion over the copper oxide - iron oxide pellets will naturally produce an expansion in volume, as the volume of carbon dioxide produced will be greater than that of the hydrocarbon.

If the paraffins are presumed to be only "methane" and "ethane" the expansion will be equivalent to the "ethane," whilst the "methane" will be equivalent to the carbon dioxide produced less twice the volume of "ethane," *i.e.*, in the above example, "ethane" would be 32·4 — 31·2 or 1·2 per cent. and the "methane" 32·4 — 9·0 — 2·4 or 21·0 per cent. Apart from the calculation of n in the paraffins, each analysis is a simple subtraction and, once the apparatus has been flushed out, an analysis, complete in percentages, can be obtained in two

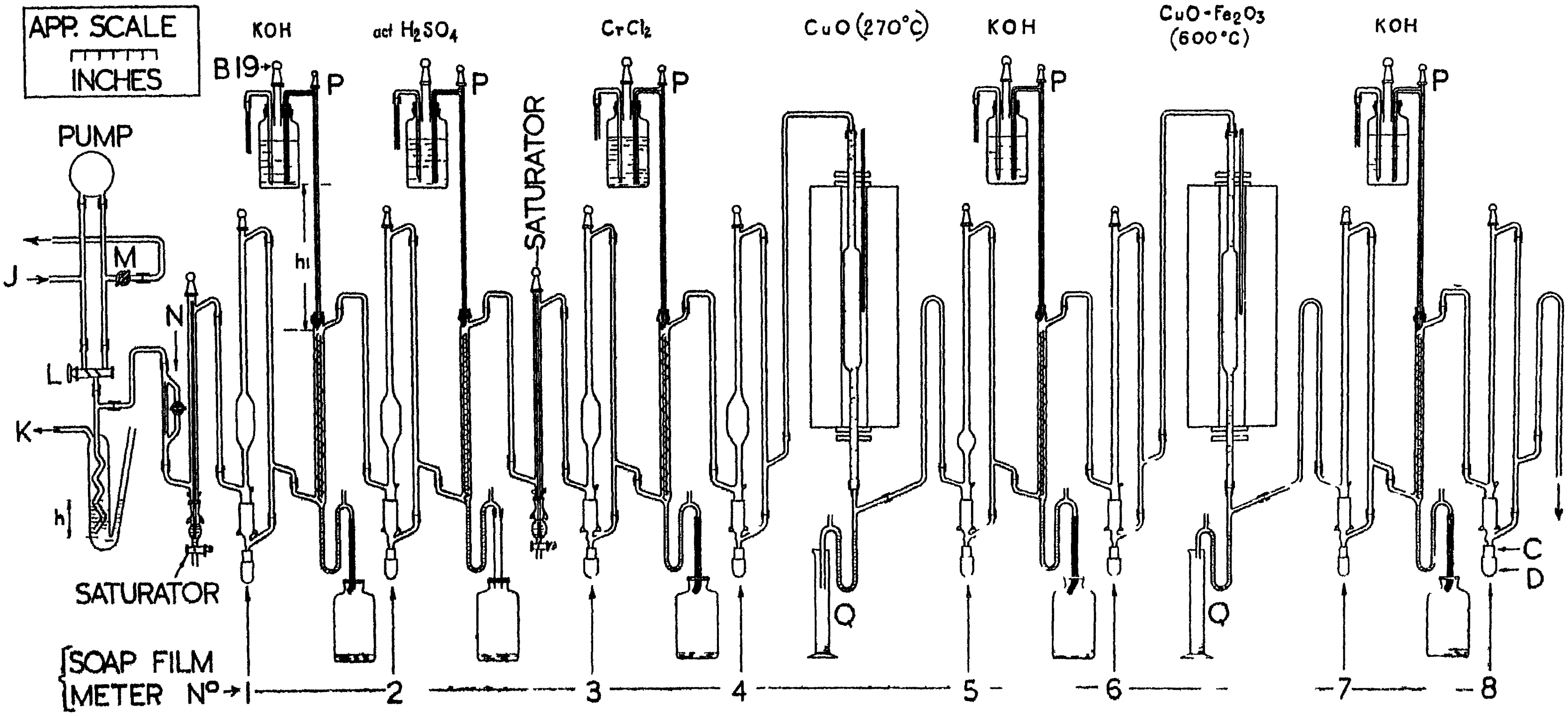
APP. SCALE
INCHES
PUMP
J
M
L
K
h
N
SATURATOR
B19
KOH
P
h1
act H_2SO_4
P
SATURATOR
$CrCl_2$
P
CuO (270°C)
Q
KOH
P
$CuO-Fe_2O_3$ (600°C)
Q
KOH
P
C
D
SOAP FILM METER No
1
2
3
4
5
6
7
8

Fig 2

to three minutes. If the concentration of nitrogen is below about 5 per cent. it is necessary to make a film in meter No. 8 first by raising C — and not D — before making the other films. In this way, a film can be got up on to the graduations in meter No. 8 for a reading, whereas the normal method of operation will leave the film below the calibration marks. Making a film in one meter independently of the others is sometimes useful in other circumstances, such as when seven meters are wet with soap, but one is not; hence the independent movement of tubes C and D is useful and necessary.

It will be realised that there are no temperature or pressure corrections. All the soap film meters are in a cabinet together and if the temperature varies it alters all volumes equally. Similarly, the whole apparatus is specifically designed to have a negligible pressure drop of about 0·2 in. (water gauge) across the whole series of meters, scrubbers and oxide pipettes.

The cups should be held firmly in the same "up" or "down" positions. When the cup is moved up, the gas in the calibrated part of the meter is displaced a little forward by the volume of soap solution. This displacement occurs twice, however, and thus cancels out when one volume is subtracted from the other, provided the cup is in the same position on each occasion. The cups must fall readily; if not, they should be weighted by addition of mercury.

It is preferable to have only the operating films present in each meter during an analysis. If, however, other films are present it should be remembered that the film being measured is always the one above the last film, since the latter is inevitably made as the film under measurement is stopped.

A useful feature of the apparatus is that, once it has been flushed out, analyses can be repeated quickly. Two or more analyses can be obtained in a few minutes before changing over to another gas stream. Other advantages of the apparatus are—greater freedom from solubility and adsorption errors, avoidance of errors due to absorption of gases by rubber joints, which can thus be freely used, less danger of breakages, no pressure or temperature corrections, no dead spaces and no taps or mercury to be cleaned. Some of these advantages are inherent in a streaming system, which, however, has disadvantages in needing more gas for analysis and in lacking the flexibility of the constant-volume and other static gas analysis apparatus. The speed and accuracy with which a gas can be analysed directly in percentages outweigh the latter disadvantages. By photographing the films in the top and bottom positions it is possible to obtain a permanent record of an analytical result.

When analyses have been completed, the reagents are stopped from flowing by taking off the caps, P, and thus breaking the syphons. Air should then be passed through the whole apparatus with the tap, N, by-passing the control capillary, wide open and with the cups up so that plenty of air passes through to reoxidise the copper without evaporating the soap solution. It has been found to be most convenient to carry out the revivification overnight.

SIZES OF METERS—

By suitable choice of meter sizes, apparatus can be made to cover all kinds of gas analysis. In the apparatus shown in Fig. 2 for the analysis of coal gas, the meters have the following approximate sizes and are based on 50 ml. ≡ 100 per cent.

	No 1	No 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8
Bottom stem:								
Intern. diam., mm. ..	8	8	8	8	8	8	8	5
Graduations % ..	0 to 8	0 to 8	0 to 8	0 to 8	0 to 4	straight tubes*		
Top stem:								
Intern. diam., mm. ..	8	8	8	8	8	8	8	5
Graduations, % ..	90 to 115	85 to 110	85 to 110	80 to 105	25 to 60	0 to 40*	0 to 40*	0 to 20*

* Over total length of tube.

These sizes are intended to give a reasonable accuracy together with a relatively short time for flushing out the apparatus. It will be realised that, with a gas containing, for example, 5 per cent. of nitrogen, the rate of flow in meter No. 8 is only 1 ml. per min. if the initial rate of flow through meter No. 1 is 20 ml. per min.; so that, if the volume of meter No. 8 were not reduced from 50 ml., the gas would take 50 minutes to flow through it. By reducing the volume to 10 ml. (*i.e.*, 20 per cent.), the time to flush out the eighth meter is decreased to 10 minutes. It will be seen that all meters are progressively reduced in size in an attempt to get approximately equal rates of flow of the films longitudinally. The apparatus is, however, capable of wide variations and with a second apparatus having the following sized meters, for the analysis of producer and waste gases, it becomes possible to analyse most types of manufactured fuel gases or waste gases. Stem. int. diam. = 8 mm.; 50 ml. ≡ 100 per cent.; bottom stems, 0 to 8 per cent.; top stems—meter No. 1, 90 to 115 per cent.; Nos. 2 to 5, 75 to 100 per cent.; Nos. 6 and 7, 50 to 80 per cent.; No. 8, 45 to 75 per cent.

It would, of course, be possible to manufacture an apparatus containing eight similarly sized and similarly calibrated meters which would analyse any type of gas Accuracy—or convenient size—would, however, have to be sacrificed and a longer time would be necessary to flush out the apparatus before an analysis could be made. With gases low in nitrogen content this time might be excessive.

Other units have been made for special purposes. A portable apparatus for the determination of carbon dioxide, oxygen and carbon monoxide was based on the following dimensions:—33 ml. ≡ 100 per cent.; bottom calibrations 0 to 8 per cent.; top calibrations for the four soap film meters, 80 to 110 per cent., 74 to 104 per cent., 71 to 101 per cent. and 71 to 101 per cent.

A small portable apparatus for the determination of carbon dioxide was based on 25 ml. ≡ 100 per cent.; so were small forms of the apparatus for the determination of oxygen and of nitrogen. In the last one, the gas, after passing through the first meter, passed over Arneil catalyst at 600° C. and soda asbestos, so that only nitrogen remained to flow in the second and much smaller meter. The solid reagent for the removal of carbon dioxide was packed in sizeable pieces (3 to 8 mesh) to avoid back pressures in a U-tube or similar vessel fitted with ground-glass tops so as to be readily removable for recharging.

An attempt was made to increase the accuracy of a simple apparatus for the determination of oxygen, by having large bulbs with narrow stems so that 0·1 per cent. was equivalent to about 7 mm. It was thus possible to obtain a second decimal place in the analytical results.

USE OF APPARATUS—

The holders described earlier (ref.[1] Figs. 4 and 5) have been found effective, but not long-lived. The thickness of the rings should be greater than the 32 S. W. G. previously specified and the outside edges should be effectively and heavily soldered. The governor (*ibid*, Fig. 6) tended to choke on the smaller sintered-glass filter and it was found better to have no filter, but to have the capillary above the variation in mercury levels. The governor has not been used much, however, as the works samples of gas were readily available in small 10 cu. ft. holders used primarily for calorimetry, while another, more conventional type of governor

TIME	CO_2 %	C_nH_m %	O_2 %	H_2 %	CO %	"C_2H_6" %	"CH_4" %	N_2 %
9.50	Start on gas from No. 1 Retort House							
11.12	3·2	1·9	0·2	57·9	14·9	2·5	—	—
11 17	3·2	1·9	nil	58·0	15·1	2·4	14·6	4.8
11.21	3·1	2·0	0·1	58·0	15·0	2·0	15 2	4·6
11.37	changed over to gas from No. 4 Retort House							
12.16	1·8	2·0	1·3	55·7	10·2	2·7	19·5	6·8
12.21	2·0	1·9	1·2	55·8	9·7	3·1	19·4	6·9
12.26	1·9	2·1	1·1	55·8	9·8	3·0	19·4	6·9
12.30	changed over to District Gas							
1.40	2·4	2·0	0·9	53·2	10·7	2·5	21·0	7·3
1.45	2·5	2·1	0·6	53·3	10·6	2·5	21·0	7·4
1.49	changed over to gas from No. 3 Retort House							
2.50	2·1	1·8	1·1	49·0	7·1	2·6	23·1	13·2
2.54	2·3	1·9	1·0	48·5	6·9	2·3	23·2	13·9
2.58	2·2	1·8	1·0	48·5	6·8	2·2	23·5	14·0
3.2	changed over to gas from No. 5 Retort House							
3.55	3·0	2·2	0·4	41·2	6·0	3·2	—	—
4.0	2·8	2·2	0·4	41·3	6·1	3 0	24·0	20·2
4.3	2·8	2·3	0·5	41·2	6·4	3·2	23·2	20·4
4.6	changed over to gas from "inlet holders"							
4·31	3·0	1·9	0·6	52·6	9.2	3·0	16·3	13·4
4.39	2·8	1·8	0·7	53·0	10·4	2·9	16·4	12·0
4.45	3·1	1·7	0·8	52·9	10·6	3·0	16·2	10·7
4.53	2·8	1·9	0·7	52·9	10·7	2·9	19·9	8·2
4.58	3·8	2·0	0·9	52·9	10·5	2·7	20·7	7·5
5.3	3·2	2·0	0·8	52·7	10·9	2·8	20·4	7·2
5.10	shut down							

was used to control the flow of gas from diaphragm holders to the gas analysis apparatus. The latter has been extensively used and it is regretted that although the modifications described above were first made in 1940, the war prevented the commercial development of the apparatus and only "home-made" models have been produced. One such model has, however, been in use in a works laboratory since November, 1941, and is still working well. As many as six different analyses have been made in one day, each analysis being repeated: the following figures give the results exactly as obtained for the analysis of six gas streams.

Another "home-made" model of an apparatus for the analysis of producer gas was effectively used in a wartime study on the production of producer gas for use in motor cars. It was the practice in this work to take certain readings of temperatures, pressures, gas flow, etc., every quarter of an hour, when, in addition, a gas analysis was made. It was thus possible to obtain many complete analyses over a long test period.

These forms of apparatus have also been used with considerable success in research work on catalysts in streaming systems. Part of the gas at the outlet of a catalyst vessel was made to flow through a soap film apparatus and, despite the fact that no nitrogen was present and thus soap films remained stationary in the last meter, successful gas analyses were continuously obtained. Other smaller forms of soap film apparatus have been used for the analysis of carbon dioxide from exhausts of internal combustion engines and in the C.A.B. Test for the combustibility of coke, for the determination of carbon dioxide, oxygen and carbon monoxide in waste gas and for the determination of oxygen.

The types of apparatus mentioned above have been fully tested by analysing known gas mixtures, by checking analyses with the constant-volume and other apparatus and by calibrating the meters with air while water flowed down the scrubbers and while the furnace tubes remained unheated.

SUMMARY

The method of gas analysis described in 1940 has been further developed.

A new type of soap-film gas meter has now made it possible to obtain the results, with repeats, of gas analyses directly in percentages within a few minutes after an initial period necessary to flush out the apparatus.

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DISCUSSION

MR. A. F. WILLIAMS asked how in the apparatus described provision was made for different times of contact necessary for different reactions, *e.g.*, the oxidation of hydrogen takes longer than the absorption of carbon dioxide.

MR. W. H. C. SIMMONS asked if the apparatus had been applied to the analysis of flue gases from boilers.

MR. E. W. MUDDIMAN enquired as to the accuracy of the apparatus and asked if any particular form of soap solution was used in it.

MR. H. N. WILSON enquired how much gas would be necessary to bring the apparatus to equilibrium and sweep out.

MR. T. A. RYSON asked if it would be of any use to dilute the gas analysed with nitrogen in order to speed up the sweep-out time.

MR. E. BISHOP enquired whether the time required for sweeping out the apparatus could be conveniently reduced by temporarily increasing the through-put of the sample.

MR. GOODERHAM, replying to these questions, stated that the combustion pipettes were of sufficient size to allow the oxidation of hydrogen, carbon monoxide and paraffins to be complete. The time necessary for sweeping out the apparatus and reaching equilibrium conditions could, in an emergency, be reduced by opening temporarily the tap by-passing the control capillary, and the agreement between results of repeat analyses would show if purging was complete. One to one and a half litres of gas were usually sufficient to complete a full analysis, with several repeats. The accuracy of the apparatus demonstrated was 0·2 per cent. on each constituent, but it might be possible to get greater accuracy with a larger ratio of bulb to stem. Apparatus for the determination of carbon dioxide, oxygen and carbon monoxide in waste gases had been made and used. A special acidic soap solution was to be preferred.

The Spectrophotometric Determination of Small Proportions of Linolenic Acid in Fats

By T. P. HILDITCH and R. K. SHRIVASTAVA

The determination of linolenic acid in a mixture of fatty acids, from the extinction-coefficient for ultra-violet absorption at 268 mμ after isomerisation with alkali under standardised conditions of concentration, time and temperature (180° or 170° C.), has been described by Mitchell, Kraybill and Zscheile[1] and by Hilditch, Morton and Riley.[2] The latter authors recommended isomerisation with alkali at 170° C. for 15 minutes in the determination of linolenic acid, and in subsequent papers Hilditch, Gunstone and Riley[3,4,5] showed further that, in mixtures of saturated, oleic, linoleic and linolenic acids, the precision of the spectrophotometric analysis is increased if the mixture is first resolved by crystallisation from acetone or from ether at low temperatures into a series of fractions, in each of which either the saturated, oleic, or polyethenoid acids are concentrated.

Meanwhile the application of this method has been reported by several investigators to reveal the presence of slight absorption ($E^{1\%}_{1\,cm.}$ of a few units, usually not exceeding 10) at 268 mμ after the standard isomerisation with alkali in fats and fatty acids in which hitherto the presence of linolenic acid has not been recorded (*cf.*, *e.g.*, Brice, Swain, Schaeffer and Ault).[6] It would appear that in some instances the occurrence of such small absorption has been credited arbitrarily to linolenic acid. We ourselves also have observed the presence, after isomerisation with alkali, of slight absorption at 268 mμ in fatty oils such as cottonseed and sunflower-seed oils, but have felt uncertain as to the propriety of calculating this to linolenic acid in the absence of independent proof that traces of the triethenoid acid were indeed present. It seemed possible either that, in presence of a large concentration of linoleic acid, the intense band at 234 mμ might be responsible for the presence of very slight absorption as far as a wave-length of 268 mμ, or alternatively, that the latter slight absorption might be caused by traces of non-fatty components (either present as such or produced by the treatment with alkali).

We have therefore examined a group of seed fatty oils, (i) by observing the value of $E^{1\%}_{1\,cm.}$ at 268 mμ after isomerisation of their total fatty acids, and (ii) by submitting the mixed fatty acids to crystallisation from 10 per cent. solutions in acetone at − 60° C. and then isomerising the soluble portion with alkali and determining its extinction-coefficient at 268 mμ. In general we have found that the apparent values for linolenic acid obtained by these two procedures were in close agreement. The apparent concentration of linolenic acid in the fraction of acids soluble in acetone at − 60° C. was, however, usually sufficient to warrant its detection as hexabromostearic acid ("linolenic hexabromide") by addition of bromine to an ethereal solution of this fraction of the total acids: but, of the oils examined, only soya-bean and niger-seed oils responded positively to this test. We are disposed to conclude, therefore, that linolenic acid is not, in fact, present in detectable quantity in the other oils, and to suggest that small values, of the order of less than 10, of $E^{1\%}_{1\,cm.}$ at 268 mμ on the total fatty acids of an oil, should not be calculated to linolenic acid unless positive identification of the latter acid has been obtained by other means.

That the identification of linolenic acid, present to the extent of only 0·3 per cent. of the total fatty acids, can in fact be achieved by means of concentration of the acid into the portion soluble in a suitable solvent (acetone) at low temperatures is illustrated by the example of palm oil, recently studied in this laboratory by Hilditch, Meara and Roels.[7] The mixed acids of a plantation palm oil (iodine value of the oil 52·9) left 13·4 per cent. of their weight as a fraction soluble in acetone (10 ml. per g.) at − 40° C. This soluble fraction had iodine value 151·0 and, after isomerisation with alkali at 170° C. for 15 minutes, gave $E^{1\%}_{1\,cm.}$ 13 at 268 mμ, corresponding to 2·4 per cent. of linolenic acid in the fraction and to 0·3 per cent. of linolenic acid in the total fatty acids of the palm oil. On treatment of an ethereal solution at 0° C. with bromine, a very small quantity of crystalline bromo-adduct was deposited, which melted at 174° C. and, when mixed with authentic hexabromostearic acid (m.p. 180°), melted at 176° C.

EXPERIMENTAL

The fatty oils studied were cottonseed, maize, sunflower-seed, sesame, groundnut (Indian and Nigerian), niger-seed, and soya-bean oils. The last-named was included since it is already accepted that linolenic acid is one of its minor components.

The mixed acids from each oil were isomerised with alkali under the conditions specified by Hilditch, Morton and Riley[2] and then examined in a Beckman spectrophotometer; the results are given in Table I.

TABLE I

$E^{1\%}_{1\,\text{cm.}}$ AT 268 mμ AFTER ALKALI-ISOMERISATION OF MIXED FATTY ACIDS

Fatty oil	Iodine value: Oil	Iodine value: Mixed acids	$E^{1\%}_{1\,\text{cm.}}$ 268 mμ	Calculated to linolenic acid %
Cottonseed	103·0	106·5	8·8	1·7
Groundnut (Indian).. ..	93·0	96·5	1·1	0·2
,, (Nigerian) ..	86·3	90·8	—	nil
Maize..	112·6	117·8	3·4	0·6
Niger seed	136·4	139·7	23·5	4·4
Sesame	113·4	116·0	3·5	0·7
Soya bean	132·6	136·7	50·1	9·4
Sunflower seed	135·7	140·7	3·4	0·6

The mixed acids from each oil were then crystallised from 10 per cent. solution in acetone at −60° C., for five hours, after which the deposited crystalline acids were removed by filtration and the weights and iodine values of both this insoluble and the soluble fractions determined. Portions of each fraction were also isomerised with alkali under the standard conditions at 170° C. for 15 minutes and at 180° C. for 60 minutes, followed by spectrophotometric determination of $E^{1\%}_{1\,\text{cm.}}$ at 268 mμ and 234 mμ respectively. Further, a portion of the soluble fatty acid fraction, in 10 per cent. solution in ether at 0° C., was treated with bromine until a faint yellow colour persisted; if any crystalline bromo-additive compound separated, it was filtered and its melting-point determined. The data for apparent linolenic acid contents thus obtained are shown in Table II, except those for niger-seed, soya-bean and sunflower-seed oils, which are dealt with in Table III.

TABLE II

APPARENT LINOLENIC ACID CONTENTS AND IDENTITY TESTS OF SOLUBLE FRACTIONS FROM MIXED FATTY ACIDS

Fatty oil	Acids soluble in 10% acetone solution at −60° C.				Bromination test
	% (of total acids)	$E^{1\%}_{1\,\text{cm.}}$ 268 mμ	Calculated to linolenic acid: In fraction %	Calculated to linolenic acid: On total acids %	
Cottonseed	33·7	23·9	4·5	1·5	Negative
Groundnut (Indian) ..	26·3	3·3	0·6	0·2	,,
,, (Nigerian) ..	18·2	0·9	0·2	Trace	,,
Maize	31·3	9·5	1·8	0·6	,,
Sesame	36·1	9·0	1·7	0·6	,,

Both the insoluble and soluble fractions of the mixed acids of niger-seed and of soya-bean oils showed absorption at 268 mμ after isomerisation with alkali. The sunflower-seed mixed acids behaved differently from the rest in that on crystallisation from 10 per cent. solution in acetone at − 60° C., for five hours, acids of higher iodine value (127·1) than in other instances were deposited. The portions insoluble and soluble at −60° C. were therefore separately crystallised again from 20 per cent. solution in acetone at −60° C., and two further insoluble fractions (A and B) obtained. The portions left in solution from these separations were combined and finally crystallised from 20 per cent. solution in acetone at −70° C. for five hours, when a fraction (C) was deposited leaving in solution a small amount of most soluble acids (D). The iodine value of this most soluble fraction (147·2) was considerably lower than that of fraction B (171·8) or C (167·8). It would seem that the high linoleic acid content of sunflower-seed oil resulted in high concentrations of linoleic acid appearing in the original portions left in solution at −60° C., and that further low-temperature crystallisation of these caused deposition of acids in which linoleic acid was still more highly

TABLE III

APPARENT LINOLENIC ACID CONTENTS AND IDENTITY TESTS

(Niger-seed, soya-bean and sunflower-seed oils)

Fatty oil		Fatty acid fractions separated by low-temperature crystallisation					Bromination test
		% (of total acids)	Iodine value	$E^{1\%}_{1\,cm.}$ 268 mμ	Calculated to linolenic acid		
					In fraction %	On total acids %	
Niger seed ..	Insoluble	76·0	134·7	10·1	1·9	4·1	Positive (m.p., 176–177°)
	Soluble	24·0	155·8	58·2	10·9		
Soya bean .	Insoluble	52·3	101·1	17·2	3.2	9·4	Positive (m.p., 183°)
	Soluble	47·7	178·0	85·6	16·1		
Sunflower seed ..	A*	51·1	117·7	—	—	0·7	
	B*	19·8	171·8	4·7	0·9		Negative
	C*	23·5	167·8	6·6	1·2		Negative
	D*	5·6	147·2	20·8	3·9		Negative

* See text above.

TABLE IV

COMPONENT FATTY ACIDS OF THE OILS

Fatty oil		Fatty acid fractions obtained by low-temperature crystallisation							
		% (of total acids)	Iodine value	$E^{1\%}_{1cm.}$ 268 mμ	$E^{1\%}_{1cm.}$ 234 mμ	Composition			
						Satd.* %	Oleic %	Linoleic %	Linolenic %
Cottonseed ..	Insoluble	66·3	80·8	—	234·0	36·5	37·7	25·8	—
	Soluble	33·7	155·8	neglected	737·9	9·6	8·9	81·5	—
Groundnut (Indian)	Insoluble	73·7	75·9	—	133·4	30·7	54·6	14·7	—
	Soluble	26·3	156·2	neglected	677·2	2·3	22·9	74·8	—
Groundnut (Nigerian)	Insoluble	81·8	76·7	—	69·2	22·7	69·7	7·6	—
	Soluble	18·2	153·0	neglected	656·3	3·5	24·0	72·5	—
Maize	Insoluble	68·7	95·7	—	237·5	20·3	53·5	26·2	—
	Soluble	31·3	165·9	neglected	735·8	—	18·8	81·2	—
Niger seed	Insoluble	76·0	134·7	10·1	633·5	24·2	5·2	68·7	1·9
	Soluble	24·0	155·8	58·2	658·2	16·1	7·2	65·8	10·9
Sesame	Insoluble	63·9	96·6	—	274·6	23·5	46·2	30·3	—
	Soluble	36·1	149·2	neglected	636·9	5·7	24·0	70·3	—
Soya bean	Insoluble	52·3	101·1	17·2	335·3	29·9	31·9	35·0	3·2
	Soluble	47·7	178·0	85·6	720·3	5·6	8·9	69·4	16·1
Sunflower seed ..	(Mixed acids)	100·0	140·7	neglected	610·1	12·2	20·5	67·3	—

Fatty oil	Composition of total fatty acids in each oil (% by wt.)			
	Saturated*	Oleic	Linoleic	Linolenic
Cottonseed	27·4	28·0	44·6	—
Groundnut (Indian)	23·2	46·3	30·5	—
,, (Nigerian)	19·2	61·4	19·4	—
Maize	14·0	42·6	43·4	—
Niger seed	22·2	5·7	68·0	4·1
Sesame	17·1	38·2	44·7	—
Soya bean	18·3	20·9	51·4	9·4
Sunflower seed	12·2	20·5	67·3	—

* The figures (obtained by difference) for saturated acids include also any unsaponifiable matter present in the fatty oils.

concentrated. The data for niger-seed, soya-bean and sunflower-seed oils are summarised in Table III.

DISCUSSION AND CONCLUSIONS

In spite of the fact that in all cases the spectrographic evidence from the mixed acids (Table I) and from the more soluble unsaturated concentrates (Tables II and III) is closely accordant, negative "hexabromide" tests were obtained with the concentrates of unsaturated acids from cottonseed, groundnut, maize, sesame and sunflower-seed oils. We therefore consider that linolenic acid cannot occur in more than minute traces in these oils, and that the small proportions indicated by spectrographic analysis should in these and similar instances be ignored. Contrariwise, there was no difficulty in confirming the presence of linolenic acid (already, of course, well established by isolation of hexabromostearic acid by many previous workers) in soya-bean oil. Finally, the occurrence of some linolenic acid, hitherto reported only in one instance (Pye[8]), in niger-seed oil was similarly proved.

We recommend, as stated earlier in this communication, that small proportions of linolenic acid should not be credited to fatty oils on the evidence of small absorption at 268 mμ after isomerisation with alkali, unless their presence can be supported by production of crystalline hexabromostearic acid from a concentrate of the unsaturated acids. Moreover, for minor proportions of linolenic acid (*e.g.*, less than 10 per cent. of the total fatty acids), we feel that the spectrophotometric method of analysis may tend in any case to record values somewhat higher than the truth, although the error is unlikely to exceed 0·5 to 1 unit per cent. of the total fatty acids.

COMPONENT FATTY ACIDS OF THE FATTY OILS

From the iodine values and spectrophotometric data of the acids obtained by low-temperature crystallisation during this investigation we have determined the proportions of saturated, oleic, linoleic and (when shown to be present) linolenic acids, with the results shown in Table IV.

The sunflower-seed and niger-seed oils were specimens the component acids of which had been examined some years previously in this laboratory by lead salt separation followed by fractional distillation of the methyl esters of the respective "solid" and "liquid" acids, unsaturated C_{18} esters being calculated as mixtures of oleate and linoleate from the iodine values of the ester fractions, with the following results:—

	Saturated %	Oleic %	Linoleic %	
Sunflower seed ..	10·5	22·0	67·5	(Hilditch and Riley[3])
Niger seed ..	11·9	17·0	71·1	(Hilditch and Sime[9])

The sunflower-seed oil figures agree fairly closely, but Hilditch and Sime's data[9] for niger-seed oil require amendment to allow for the small proportion of linolenic acid now shown to be present. Of other reports on niger-seed oil, only the recent (1945) study by Pye,[8] who determined the component acids from the iodine and thiocyanogen values of the oil, discloses the presence of linolenic acid:—saturated acids 9·6, oleic 16·3, linoleic 72·4, linolenic 1·7 per cent.

The specimens of the other seed oils used in this investigation were not examined by the more detailed ester-fractionation technique, but the values for their component acids (Table IV) recorded from the spectrophotometric examination of the insoluble and soluble acids obtained by crystallisation from acetone at − 60° C. are very similar to those recorded by various workers who have at different times applied the more detailed procedures to the respective fatty oils.[10]

The groundnut oils used in the present study were of some interest in that they represented approximately the extreme limits of iodine value encountered in genuine Indian or West African groundnut oils. It is interesting to note that the component acids observed for the two specimens lend further support to the view[11] already held that groundnut oils cover the following general range in their component acids:—

	%	%
Saturated	15–18	18–23
Oleic	66–60	56–50
Linoleic	19–22	25–27

When the proportion of oleic acid is lower, that of linoleic as well as that of the total saturated acids is generally higher, the increase being, however, somewhat more marked in the case of linoleic acid.

SUMMARY

1. Although spectrophotometric analysis discloses small absorption at 268 mμ after isomerisation of polyethenoid C_{18} acids by alkali, no evidence has been obtained of the presence of linolenic acid (as revealed by formation of ether-insoluble hexabromostearic acid, m.p. 180°) in unsaturated acid concentrates from cottonseed, groundnut, maize, sesame and sunflower-seed oils. It is concluded that linolenic acid is present, if at all, only in minute traces in these fatty oils.

2. It is recommended that small spectrophotometric values (after alkali isomerisation) not exceeding $E^{1\%}_{1\,cm.}$ 10 at 268 mμ should not be calculated as linolenic acid in a fatty oil, unless confirmed by production of crystalline hexabromostearic acid from a concentrate of the unsaturated acids of the oil.

3. Owing to extraneous absorption, linolenic acid proportions dependent upon small values of $E^{1\%}_{1\,cm.}$ at 268 mμ probably tend in any case to be somewhat higher than the true figures.

4. In niger-seed oil, the procedure recommended in this paper has established the presence of small proportions of linolenic acid (probably of the order of 3–4 per cent. of the total fatty acids of niger-seed oil).

We are indebted to Messrs. J. Bibby & Sons, Ltd., for the provision of the two groundnut oils and certain of the other seed oils used in this work; and we wish also to thank Professor R. A. Morton for placing a Beckman spectrophotometer at our disposal for the spectrographic measurements. One of us (R. K. S.) thanks the Government of India for a grant held by him during the progress of the work.

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The Determination of the Composition and Constitution of Ammonium Phosphomolybdate and the Conditions affecting its Precipitation

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AMMONIUM phosphomolybdate was first prepared by Berzelius, and it is almost exactly a hundred years since it began to attract the attention of chemists.

The history of the compound may be divided into a number of periods. It was recognised from the first that phosphorus was necessary to the formation of the yellow precipitate, but the very small proportion of the element present (about 1·7 per cent.) caused some chemists to doubt whether it was an essential constituent of the pure compound[1,2]; after about ten years it was generally agreed to be so.[3,4]

The next twenty-five years were devoted to attempts to establish the exact atomic ratio of molybdenum to phosphorus: this was finally accepted as 12 to 1, but more through faith in the Law of Definite Proportions than from reproducible experimental results.[5–9]

In the 1880's were published the two classical contributions to the subject.[10,11] Henry Pemberton became the father of a long line of very popular volumetric methods based on ammonium phosphomolybdate, while Fritz Hundeshagen, studying the compound in a more academic way, expressed his belief in the constancy of composition of the substance under the most varied conditions of precipitation. He put forward two formulae, $(NH_4)_3PO_4.12MoO_3.2HNO_3.H_2O$ for the precipitate dried over calcium chloride and potassium hydroxide for a few days and $(NH_4)_3PO_4.12MoO_3$ for the precipitate dried at 130° to 150° C.; these have been accepted by practically all subsequent workers.

There is a kind of paradox about the great influence exerted by these two chemists on their successors, for Pemberton was by no means a precise experimenter, and Hundeshagen gave no definite experimental evidence in support of his two formulae.

From that time forward, progress in this field has followed two streams. Practising analysts have constantly improved the old methods and techniques and invented new ones,[12–18] and their work includes much incidental study of some of the factors involved in precipitation and subsequent treatment but with much disagreement on details, for example the influence of chloride ion[19–22] and organic acids.[23–27] At intervals during the same period there have been a few more fundamental investigations of the chemical processes involved.[28–35] In these a serious attempt has been made to decide how many experimental factors influence the course of precipitation and the composition of the precipitate, followed by experiments in which these conditions were varied in a systematic manner. The work of Chesneau,[31] Clarens,[32] Clennell,[33] and Kitajima[35] deserves special mention. Recent work[36] on this and similar compounds, using X-ray crystallographic methods, has shown that the compound is really $(NH_4)_3\,P(Mo_{12}O_{40})$ and is more correctly named ammonium molybdophosphate. The old formulae and name are retained in this paper as being more familiar.

OBJECTS OF THE INVESTIGATION—A weakness of nearly all previous researches, even the most thorough, has been to neglect full analysis of phosphomolybdate precipitates and to place too much reliance on the weight of the precipitate or on alkalimetric titration. It has been the aim of the present work to investigate the precipitation under a variety of conditions, altering one factor at a time while maintaining others constant; and to analyse all precipitates by determination of molybdenum, phosphorus, and ammonium.

CHOICE OF METHODS FOR ANALYSING THE PRECIPITATES—In the choice of methods the chief difficulty encountered was due to the great disparity between the proportions of molybdenum (about 60 per cent.) and those of phosphorus and ammonium (1·7 and 1 to 2·4 per cent. respectively) present in the dried ammonium phosphomolybdate precipitates. In view of the fact that these are hygroscopic, it was thought best to dissolve the whole and use aliquot portions of the same solution for all determinations; this procedure involved the use of semi-micro methods for phosphorus and ammonium.

Phosphorus—There is a paucity of good methods for determination of phosphorus even on the macro scale. The most famous, using magnesium pyrophosphate, suffers in accuracy from the small molecular weight of this compound as well as from other causes. For this reason, Dyer, fifty-five years ago, could already speak of "this old-fashioned method."

Search was made in the literature for less common methods, and that of Cattelain and Chabrier[37] was tried. In this method lead phosphate is precipitated and washed and then dissolved in a measured quantity of N nitric acid.

$$Pb_3(PO_4)_2 + 6HNO_3 \rightleftharpoons 3Pb(NO_3)_2 + 2H_3PO_4$$

The authors state that this is a balanced reaction, but it is carried to completion by addition of N sulphuric acid, lead sulphate being precipitated. The excess of strong acids and the first stage of the liberated phosphoric acid are titrated with 0·1 N sodium hydroxide to the methyl orange end-point and titration is then continued to the phenolphthalein end-point, giving the second phosphoric stage.

This method was tried first on a scale one-tenth that described. After much work, and substitution of bromocresol green and mixed phenolphthalein and α-naphtholphthalein for the older indicators, it was finally concluded that the method is not accurate on this scale (if, indeed, it is on the usual scale). The second end-point is quite indefinite, doubtless owing to metathesis between the lead sulphate precipitate and the sodium phosphate in solution.

Though this line of attack failed, it suggested the determination of phosphorus gravimetrically as lead phosphate, which is probably the least soluble of all phosphates and certainly one of the heaviest. Conditions of precipitation were investigated and it was found that the theoretical amount of lead acetate reagent added to a given amount of KH_2PO_4 solution gave less than the theoretical amount of precipitate expressed as $Pb_3(PO_4)_2$. Addition of excess of reagent increased the amount of precipitate up to a maximum at about 33 per cent. excess. This maximum does not seem to correspond to any simple stoicheiometric proportion, and the use of an empirical factor was adopted.

Molybdenum—For determination of molybdenum the Jones reductor method was tried first, dissolving precipitates in ammonia and acidifying with sulphuric acid. This involves reduction of Mo^{VI} to Mo^{III} by passage of the acid solution through a specially packed column of amalgamated zinc into a ferric iron solution; an amount of iron equivalent to the Mo^{III} is reduced to the ferrous state and determined by titration against standard permanganate or dichromate. In spite of claims to the contrary by other analysts, the method appeared to be unsatisfactory. By the use of weaker acids it was found that while the highest *p*H that allowed satisfactory reduction without side-reactions was 1·6,* reprecipitation of phosphomolybdate began when its ammoniacal solution was brought to *p*H 1·7* by addition of acid.

Determination of molybdenum by means of 8-hydroxyquinoline (oxine)[38,39] was found to be completely satisfactory, and was adopted.

Ammonium—Determination of ammonium by addition of sodium hydroxide, distillation, and micro-titration was finally rejected after very extensive trials. The precision was not satisfactory and much trouble was experienced through puncturing of the micro-still by the hot alkali.

In spite of the criticisms of Kolthoff and others,[40–42] the hypobromite method was found to be completely satisfactory, even in presence of molybdenum; it is also rapid and convenient. The ammonium is oxidised to nitrogen by the hypobromite and the excess of this caused to liberate iodine, which is titrated with thiosulphate.[43] A semimicro-scale version of the method was worked out and used in the analysis of phosphomolybdate precipitates (see p. 534).

The various methods of analysis adopted were shown to be accurate when applied to known test-solutions (see Table I). Their aplication to the analyses of phosphomolybdate precipitates is described in detail in the following section.

TABLE I

TESTING OF ANALYTICAL METHODS

MOLYBDENUM—("Oxine" gravimetric method)—

Tested on ammonium molybdate solution (10 ml.):

Mo present: 0·1370 g.
" found: 0·1367 to 0·1369 (seven determinations) mean value *0·1368*.

AMMONIUM—(Oxidation by hypobromite, volumetric method)—

Tested on ammonium chloride solution (5 ml.):

found: 98·8, 98·4, 98·7, 97·8; mean *98·4*% of the theoretical value.

Tested on ammonium molybdate solution (5 ml.):

found: 99·0, 99·6; mean *99·3*% of the theoretical value required by $(NH_4)_6Mo_7O_{24}.4H_2O$.

PHOSPHORUS—("Lead phosphate" gravimetric method)—

Tested on potassium dihydrogen phosphate (0·0160 g.):

weight of precipitate: 0·0516 g.
0·0518
0·0519 } mean *0·0517* g.
0·0519
0·0515

empirical factor based on this mean value:—
P/"Lead phosphate" = 0·07045.

Several incidental investigations were involved in this search for analytical methods and are here briefly mentioned. The time and temperature needed to dry Gooch crucibles to constant weight, the conditions of use of desiccators of various kinds, and several methods of washing phosphomolybdate precipitates, were investigated with some care. Although the acidimetric methods for ammonium and phosphorus were finally rejected, considerable time was spent in a search for the most suitable indicators.

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* Calculated values.

PROCEDURE USED FOR THE ANALYSIS OF PHOSPHOMOLYBDATE PRECIPITATES

In most of the experiments recorded in Tables II to IV the phosphomolybdate precipitates were obtained from the same quantity of potassium dihydrogen phosphate, 0·0320 g., and in the procedure described below the quantities of reagents mentioned are, except where otherwise stated, those found convenient for that quantity of precipitate.

The precipitate, contained in a Gooch crucible, was dissolved in 20 ml. of *N* sodium acetate (*p*H 8·7) by standing the crucible in a filter funnel in the neck of a 100-ml. graduated flask and allowing portions of the acetate solution, alternating with water, to drain through, dissolution being assisted by stirring and scrubbing the walls of the crucible with a thin glass rod. The solution was then diluted to the mark, mixed and filtered (Solution A).

For the determination of ammonium, a buffered hypobromite solution was prepared, twice daily, by adding to 10 ml. of 0·1 *N* bromine, or the equivalent acidified potassium bromide and bromate solution, sufficient sodium hydroxide solution to give a lemon-yellow colour, and after 1 hour adding 25 ml. of a borate buffer solution of *p*H 8·8 (prepared by dissolving 87·5 g. of boric acid and 14·6 g. of sodium hydroxide in 800 ml. of water, boiling for half an hour to remove any trace of ammonia and diluting to 1 litre). After addition of the borate buffer the hypobromite solution was diluted to 100 ml. with water freed from carbon dioxide. Ten ml. of this buffered hypobromite reagnet were added to 5 ml. of Solution A in a 100-ml. stoppered bottle, and after standing for 10 minutes in the dark, the mixture was treated with 1 g. of potassium iodide and 5 ml. of 2·5 *N* hydrochloric acid and the liberated iodine was titrated with 0·01 *N* sodium thiosulphate in presence of starch indicator. With each batch of tests a blank determination was carried out with 5 ml. of water containing the same amount of sodium acetate, in place of 5 ml. of Solution A. Results obtained by this method on standard solutions of ammonium chloride and ammonium molybdate are shown in Table I. One ml. of 0·01 *N* thiosulphate ≡ 0·0000601 g. of NH_4.

For the determination of molybdenum and phosphorus, 50 ml. of Solution A were diluted with 50 to 75 ml. of water and heated just to boiling. The flame was then removed and molybdenum was precipitated by dropwise addition, with continuous stirring, of a 2 per cent. oxine reagent prepared by dissolving 5 g. of 8-hydroxyquinoline in 22·3 ml. of glacial acetic acid diluted with a little water, adding a solution of 17·0 g. of sodium acetate and diluting the mixture to 250 ml. with water. The dropwise addition of the reagent was continued until a slightly yellow colour was imparted to the liquid and then about 0·5 ml. of the reagent was added in excess. The total volume of oxine reagent required for most of the phosphomolybdate precipitates analysed was 21·7 ml. After the addition of the reagent, the liquid was boiled gently for 3 minutes, with stirring, and the precipitate was collected in a tared Gooch crucible, washed 6 times on the filter with hot water and dried to constant weight at 130° to 140° C. Weight of precipitate × 0·2305 = weight of molybdenum. Some results obtained on standard solutions of ammonium molybdate are shown in Table I.

The filtrate and washings from the molybdenum precipitate were adjusted to approximately *p*H 5·8 by addition of *N* sodium hydroxide. The volume required was 30 ml., provided that 21·7 ml. of the oxine reagent had been used for the molybdenum precipitation. When, owing to lower molybdenum contents, the volume of oxine reagent used was less than 21·7 ml. it was found convenient to make up the difference between the volume used and 21·7 ml. by addition of an "oxine buffer solution" similar in composition to the oxine reagent except that it contained no oxine. This ensured the presence of the same amount of acetate whatever the volume of oxine reagent used, so that the filtrate from the molybdenum precipitate could always be adjusted to *p*H 5·8 by addition of 30 ml. of *N* sodium hydroxide. After this adjustment had been made, the liquid was filtered and, at a volume of about 300 ml., heated to incipient boiling in a beaker. The flame was then removed and the phosphate was precipitated by addition of a solution containing 3 g. of AnalaR lead acetate in a litre of 0·01 *N* acetic acid; the addition was made dropwise from a burette, with continuous stirring, over a period of 10 to 15 minutes. Where, owing to low phosphate content, less than 30 ml. of the lead acetate solution was used, the deficiency was made up with 0·01 *N* acetic acid, so that the *p*H during precipitation would always be the same. After precipitation, the liquid was heated at 90° to 100° C. for about half an hour and set aside overnight, and then the precipitate was collected in a Gooch crucible, washed 6 times with hot water and dried to constant weight at 130° to 140° C. Weight of precipitate × 0·07045 = weight of phosphorus. This is an empirical value. The quantity of lead acetate solution used for precipitation is about 33 per cent. greater than the amount calculated for the formation of $Pb_3(PO_4)_2$ (*cf.* p. 533).

FORMATION AND TREATMENT OF PHOSPHOMOLYBDATE PRECIPITATES

The methods of analysis outlined above were used in a study of the conditions suitable for the formation and treatment of phosphomolybdate precipitates with a view to their determination by weighing; among the factors investigated were the influence of the quantity of precipitant used, the temperature of precipitation, the nature of the washing solutions used and the conditions of drying.

TABLE II

PHOSPHOMOLYBDATE PRECIPITATES

Obtained from the same weight (0·032 g.) of KH_2PO_4 by precipitation with different amounts of reagent
Washed with acid potassium nitrate solution

				Analysis of precipitates				
Expt. No.	Reagent volume ml.	Mo/P moles, taken	Ppt. obtained g.	Mo ppt., g.	P ppt., g.	Mo/P moles	NH_4 thio., ml.	Mo/NH_1 moles
1	10	9·4		no precipitation occurred				
2	15	14·0	0·0063					
3	„	„		0·0220			0·12	13·3
4	„	„		0·0279			0·13	15·5
5	„	„		0·0253	0·0025	10·7	0·19	9·6
6	„	„		0·0220	0·0039	6·0	0·17	9·4
7	20	18·7	0·0932					
8	„	„	0·1722	0·2232	0·0194	12·4	1·71	9·4
9	„	„		0·1567	0·0124	13·4	1·35	8·4
10	„	„		0·1433	0·0118	12·8	1·25	8·3
11	„	„		0·1511	0·0151	10·6	1·21	9·0
12	„	„		0·0916	0·0091	10·6	0·72	9·2
13	25	23·4	0·2770					
14	„	„		0·4535	0·0395	12·1	3·00	10·9
15	„	„		0·4303	0·0367	12·4	2·85	10·9
16	30	28·1	0·3326					
17	„	„		0·4420	0·0401	11·6	3·57	9·0
18	„	„		0·4501	0·0397	12·0	3·44	9·5
19	35	32·8	0·4521					
20	„	„	0·4512	0·5776	0·0506	12·1	3·50	11·9
21	„	„		0·5674	0·0501	12·0	3·45	11·9
22	„	„		0·5714	0·0502	12·0	3·12	13·2
23	40	37·4	0·4475	0·5716	0·0508	11·9	3·58	11·5
24	„	„	0·4486	0·5776	0·0507	12·0	4·08	10·2
25	„	„		0·5736	0·0502	12·1	3·90	10·6
26	„	„		0·5801	0·0511	12·0	3·74	11·2
27	„	„		0·5543	0·0490	11·9	3·60	11·1
28	„	„		0·5668	0·0499	12·0	3·51	11·7
29	45	42·1	0·4554					
30	„	„		0·5842	0·0526	11·7	3·47	12·2
31	„	„		0·5740	0·0500	12·1	3·56	11·7
	50	46·8	0·4559	0·5829	0·0509	12·1	3·69	11·6
			(mean value of five precipitates)					Theory = 4

Formation of phosphomolybdate precipitates—The method of precipitation was in the first place chosen arbitrarily after a thorough study of the best known methods; it was modified somewhat in the light of experience but its main features were justified by the results obtained.

AnalaR potassium dihydrogen phosphate was used as the source of phosphorus. Being subject to mould growth, its solutions were prepared afresh every 2 or 3 weeks.

The acid ammonium molybdate reagent was prepared in accordance with a formula kindly supplied by Dr. A. D. Mitchell (see Appendix). It is very stable and develops no turbidity on being heated for several hours at 57° C., even in presence of molybdic acid nuclei.

Precipitation was carried out in a large water bath maintained at constant temperature. The phosphate solution mixed with 8 ml. of concentrated nitric acid, 100 ml. in all, was contained in a beaker and the reagent (50 ml.) was added in a fine stream from a burette, with continuous stirring. The time of outflow was 7 minutes. The mixture was kept at the bath temperature for 75 minutes and then allowed to cool for 60 minutes.

Filtration and washing—The precipitates were washed three times by decantation, collected in Gooch or sintered-glass crucibles and washed six times more in the crucibles.

In the earlier experiments, including all those summarised in Tables II and III, the washing solution used was one containing 0·5 per cent. of nitric acid and 1 per cent. of potassium nitrate; as ammonium was to be determined in the precipitates it was not considered advisable to have an ammonium salt in the washing solution. The results indicated, however, that the potassium in the washing solution displaced most of the ammonium from the precipitate. In later experiments, therefore, a washing solution containing 0·5 per cent. of nitric acid and 0·8 per cent. of ammonium nitrate was used, and a final wash was given with a 1 per cent. solution of nitric acid. This method of washing was used in the experiments summarised in Table IV and is recommended in the Appendix.

TABLE III

PHOSPHOMOLYBDATE PRECIPITATES

Obtained from the same weight (0·032 g.) of KH_2PO_4 by precipitation at various temperatures
Washed with acid potassium nitrate solution

			Analysis of precipitates				
Expt. No.	Pptn. temp. ° C.	Ppt. obtained g.	Mo ppt., g.	P ppt., g.	Mo/P moles	NH_4 thio., ml.	Mo/NH_4 moles
1	15	0·4342	0·5570	0·0491	12·0	3·76	10·7
2	„		0·5154	0·0436	12·5	2·86	13·0
3	„		0·5279	0·0455	12·3	2·87	13·3
4	30	0·4374					
5	„		0·5701	0·0501	12·0	3·17	13·0
6	„		0·5801	0·0513	12·0	2·91	14·4
7	40	0·4488					
8	„		0·5806	0·0504	12·2	3·49	12·0
9	„		0·5795	0·0507	12·1	3·55	11·8
10	50	0·4568	0·5861	0·0511	12·1	3·86	11·0
11	„	0·4550	0·5824	0·0503	12·2	3·46	12·2
12	„	0·4560	0·5832	0·0506	12·2	3·47	12·1
13	„		0·5831	0·0510	12·1	3·85	10·9
14	„		0·5836	0·0516	11·9	3·61	11·7
15	„		0·5824	0·0505	12·2	3·88	10·9
16	61	0·4564					
17	„		0·5869	0·0516	12·0	3·22	13·2
18	„		0·5839	0·0513	12·0	3·40	12·4
19	70	0·4550	0·5833	0·0507	12·2	3·77	11·2
20	„		0·5865	0·0504	12·3	4·13	10·3
21	„		0·5867	0·0506	12·3	4·26	10·0
22	77	0·4622					
23	„		0·5916	0·0520	12·0	4·54	9·4
24	„		0·6085	0·0518	12·4	4·61	9·5
		Mean ratios (all)	..	.. ..	12·15	.. ..	11·7
		„ „ (50° C.)	..	.. ..	12·1	.. ..	11·5

Estimations of the solubility of freshly prepared phosphomolybdate precipitates in various possible washing liquids were made. In each test, the precipitate from 0·032 g. of potassium dihydrogen phosphate, after being washed with the particular solution under test, was transferred to a large volume of similar solution and left for several days, with occasional shaking. The clear filtered extract was evaporated to small volume and, if acid, neutralised with sodium hydroxide solution. After buffering with 10 ml. of *N* sodium acetate, determinations were made of molybdenum and phosphorus by the methods described on p. 534. From the molybdenum values the weights of phosphomolybdate dissolved by 100 ml. of the solutions tested were calculated. They are as follows and are averages of closely concordant replicate results.

Solution containing 1% of HNO_3—26·2 mg. per 100 ml.
„ „ 0·5% of HNO_3 and 1% of KNO_3—6·9 mg. per 100 ml.
„ „ 0·8% of NH_4NO_3—6·2 mg. per 100 ml.
„ „ 0·5% of HNO_3 and 0·8% of NH_4NO_3—0·8 mg. per 100 ml.

An attempt was made to estimate the losses actually incurred in washing with the acid ammonium nitrate and nitric acid solutions as recommended in the Appendix. The washings (except the first) from 30 precipitates, 2560 ml. in all, were evaporated to 60 ml. and the molybdenum and phosphorus present were determined as before. The former indicated 26·5 mg. and the latter 32 mg. of phosphomolybdate, *i.e.*, a loss of about 1 mg. of precipitate (≡ 0·016 mg. of phosphorus) per determination.

Drying of precipitates—Seven methods of drying were used, five at room temperature in desiccators, with or without evacuation, and two in an electric oven, at 115° C. or at 130° to 140° C. The oven method at 130° to 140° C. was adopted for most of the determinations. Sintered-glass crucibles were dried for 2½ hours and Gooch crucibles for 8 hours at this temperature, and the same times were used in taring the empty crucibles. Both kinds were allowed to cool for 25 minutes in small desiccators containing sulphuric acid, one crucible to each desiccator. Weighing was as rapid as possible, with pre-selected weights.

The main results of the investigations outlined above are summarised in Tables II to IV.

TABLE IV

PHOSPHOMOLYBDATE PRECIPITATES

Obtained under various conditions

Washed with acid ammonium nitrate solution

A. Air-drying at 130° C.

KH_2PO_4 taken g.	Ppt. obtained g.	Analysis of precipitates: Mo ppt., g.	Mo %	P ppt., g.	P %	Mo/P moles	NH_4 thio., ml.	NH_4 %	
(7) 0·032	0·4401	0·5853	61·32	0·0522	1·67	11·85	8·89	2·41	Mo/NH_4=4·8 Theory = 4
(4) 0·032*	0·4401	0·5844	61·23	0·0517	1·65	11·95	8·88	2·41	
(3) 0·016	0·2213	0·2961	61·68	0·0270	1·72	11·6	4·32	2·35	
(4) 0·0064	0·0882	0·1206	63·0						
(3) 0·0032	0·0446	0·0621	64·3						

B. Drying *in vacuo* at room temperature

0·032 g. of KH_2PO_4 taken in all cases

Desiccant	Ppt. obtained g.	Mo ppt., g.	Mo %	P ppt., g.	P %	Mo/P moles	NH_4 thio., ml.	NH_4 %
(1) H_2SO_4	0·4415	0·5865	61·26	0·0522	1·67	11·9	8·79	2·39
(2) P_2O_5	0·4414	0·5854	61·14					
(4) H_2SO_4 & KOH	0·4413	0·5842	61·08	0·0517	1·65	11·95	8·81	2·40

Precipitate allowed to stand overnight at room temperature before filtration.

STATEMENT AND DISCUSSION OF RESULTS

Table II—These results were obtained with varying amounts of molybdate reagent, all other conditions remaining the same. They show:

(*a*) the necessity for at least 3·5 times the theoretical amount of molybdate for complete precipitation;
(*b*) that although precipitation is incomplete with less than this amount, the molybdenum/phosphorus ratio in the precipitates remains practically constant.
(*c*) that washing with potassium nitrate solution causes base exchange; apparently two of the three ammonium ions are replaced by potassium ions.

Table III—The only variable here is temperature of precipitation. The following points emerge:

(*a*) precipitation is incomplete below 50° C.;
(*b*) the ratio molybdenum/phosphorus in the precipitates is constant for all temperatures of precipitation within the range examined;
(*c*) decomposition of the reagent and co-precipitation of molybdenum occur above 70° C.;
(*d*) the base exchange phenomenon noted previously is confirmed.

Table IV (*A*)—this shows the effect of decreasing the amount of phosphorus to be precipitated while maintaining constant the amount of molybdate reagent and other factors. The bracketed numbers on the left indicate the number of replicate determinations made. The results show:

(*a*) that although the extreme variation in the molybdenum content of the precipitates is about 5 per cent., the variation in weight of the precipitates is much less (not more than 1·5 per cent.);

(*b*) that the mean weight of precipitate obtained from 0·032 g. of KH_2PO_4 is close to the theoretical value, 0·4412 g., calculated from Hundeshagen's second formula, and that the weighted mean of all the 21 results shown is 0·4415 g. calculated to the same basis;

(*c*) that the mean weight of molybdenum oxinate corresponding to 0·032 g. of KH_2PO_4 is 0·5850 g. (theoretical value 0·5873 g.);

(*d*) that although the precipitates were washed with acid ammonium nitrate solution the amount of ammonium ion found in these precipitates is only about 5/6th of the theoretical, suggesting that the precipitate may be $(NH_4)_3PO_4.(NH_4)_2HPO_4.24MoO_3$; this is surprising in view of the apparent agreement with Hundeshagen's formula in (*b*) and (*c*) above;

(*e*) that a longer time of standing has little or no influence on the amount or composition of the precipitate (second line of Table).

Table IV (*B*)—This gives a few results obtained on precipitates dried by various desiccator methods: the results are close to those obtained by heat-drying, any slight differences being too small to be of stoicheiometric significance. This raises the question whether Hundeshagen's first formula, including the nitric acid and water addenda, is correct; the point has not yet been tested directly.

CONCLUSIONS

Taking theoretical aspects first, the data obtained cast some doubt upon the correctness of Hundeshagen's famous formulae, but in view of the readiness with which base exchange occurs during washing with quite dilute solutions, it is impossible to speak with certainty on this point.

The practical conclusions are that phosphorus can be accurately determined gravimetrically as ammonium phosphomolybdate if precipitation is carried out at 50° C., using at least 3·5 times the theoretical amount of molybdate reagent, and if suitable methods of washing and drying are employed; and that determination of the molybdenum in the precipitate as oxinate provides a check which is superior in convenience, speed and accuracy to the usual magnesium pyrophosphate method.

Attempts have also been made to find a method more economical of molybdenum, but so far without success.

The influence of foreign substances on precipitation has not yet been investigated.

APPENDIX

PROCEDURE RECOMMENDED FOR THE DETERMINATION OF PHOSPHATE IN AN UNKNOWN SOLUTION

Reagents—

1. *Concentrated nitric acid* (AnalaR).

2. *Acid molybdate solution*—Dissolve 35 g. of finely powdered AnalaR ammonium molybdate in a mixture of 50 ml. of "0·880" ammonia and 50 ml. of water. Dilute 168 ml. of concentrated nitric acid with water to 360 ml. and allow to cool. Pour the molybdate solution into the nitric acid in a thin stream and with constant stirring or swirling. Very little or no precipitation should occur. Allow to stand at least 24 hours, dilute to 900 ml. with water and filter. Preserve in wax-lined bottles to prevent formation of silicomolybdate. If any precipitate develops later, filter immediately before using.

3. *Acid ammonium nitrate wash liquid*—Dissolve 20 g. of AnalaR ammonium nitrate in water, add 12·5 ml. of concentrated nitric acid and make up to 2·5 litres.

4. *Nitric acid wash liquid*—Dilute 25 ml. of concentrated nitric acid with water to 2·5 litres.

Filter crucibles—Use either the finest grade sintered glass crucible or a Gooch crucible prepared as follows. Half fill with the usual asbestos suspension, well-mixed; drain slowly and apply gentle suction. Relax the suction and pour in a little more of the suspension, after allowing the coarser particles to settle; apply strong suction. In this way a filter surface of fine grain is obtained. Wash five or six times with the nitric acid wash liquid, wipe the outside of the crucible and dry to constant weight at 130° C., cooling in a sulphuric acid desiccator 25 minutes before weighing.

To prepare sintered-glass crucibles, wash once or twice with the nitric acid and dry as above.

Both kinds of filter are readily cleaned with dilute ammonia after use.

Procedure—The phosphate solution should contain not more than 0·007 g. of phosphorus and its volume should not exceed 90 ml. Add 8 ml. of concentrated nitric acid, dilute with water to 100 ml. and heat to 50° ± 1° C. in a water bath. If this is large enough and if a shielded flame is used there is no need for thermostatic control. Add the molybdate reagent (50 ml.) from a burette slowly (time of outflow 7 to 10 minutes), with constant stirring. This allows for at least 3·5 times the theoretical amount of molybdenum. Allow the mixture to remain in the water bath at the same temperature for 75 minutes. Remove, stir once, and allow to stand in a cool place for at least 1 hour.

Decant the mother liquor into the filter as completely as possible. Add to the precipitate about 15 ml. of the ammonium nitrate wash liquid and allow to stand about 5 minutes, stirring 4 to 5 times during that period; allow to settle and decant again. Wash by decantation twice more with 10-ml. portions, allowing short periods of standing and stirring. Transfer the precipitate to the filter, wash 6 times with the same wash, filling the crucible half-full each time and using gentle suction. Suck as dry as possible after the last wash. Wash once with the nitric acid wash liquid, again filling about half full, and finally with about 1 ml. of the same solution. Suck dry, wipe the outside of the crucible, and dry at 130° to 140° C. for at least 4 hours (8 hours if a Gooch crucible is used), cool for 25 minutes in a sulphuric acid desiccator and weigh as rapidly as possible. Heat again until constant weight is attained, pre-selecting the weights to ensure rapidity of weighing; the precipitate is very hygroscopic.

The weight of precipitate multiplied by *0·01654* gives the weight of phosphorus present in grams.

N.B.—The acid in the desiccator must be kept concentrated and colourless. This is not difficult if the times of opening to insert or remove crucibles are kept as short as possible.

The work recorded in this paper was carried out in the Frankland Laboratories at the University of Birmingham.

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MERCHANT VENTURERS TECHNICAL COLLEGE
BRISTOL, 1

DISCUSSION

The PRESIDENT congratulated the author on a very useful piece of work dealing with one of the most widely used of analytical methods. He asked the author what advantages the weighing of the phosphomolybdate precipitate had over its determination by a volumetric method.

Mr. A. H. EDWARDS said that during the standardisation of a method, now accepted as a British Standard, for the determination of phosphorus in coal and coke ash, he had determined the factor required to convert ml. of 0·1 *N* sodium hydroxide to phosphorus. The factor, 1 ml. of 0·1 *N* sodium hydroxide ≡ 0·000135 g. P, which was found by precipitating ammonium phosphomolybdate from disodium hydrogen phosphate, agreed with that calculated from the equation

$$2[(NH_4)_3PO_4.12MoO_3] + 46NaOH \rightarrow 2Na_2HPO_4 + 21Na_2MoO_4 + 3(NH_4)_2MoO_4 + 22H_2O.$$

The phosphomolybdate precipitate was washed with 1 per cent. potassium nitrate solution and it seemed that under these conditions no exchange of potassium for ammonium radicals occur, otherwise a different conversion factor would have been obtained. Hundeshagen's formula for ammonium phosphomolybdate, $(NH_4)_3PO_4.12MoO_3.2HNO_3.2H_2O$, was, therefore, probably correct, and during washing with neutral potassium nitrate solution the nitric acid and water were removed from the precipitate.

Mr. THISTLETHWAITE, in reply to the President, claimed that alkalimetric methods involve more variable factors—for example, uncertainty as to the exact number of phosphoric hydrogen ions replaced, possible loss of ammonia, interference by carbon dioxide—than his gravimetric method.

In reply to Mr. Edwards, he pointed out that the equation—

$$2\ [K_2NH_4PO_4.12MoO_3] + 46NaOH \rightarrow 2Na_2HPO_4 + 21Na_2MoO_4 + 2K_2MoO_4 + (NH_4)_2MoO_4 + 22H_2O,$$

which assumes the base exchange indicated by his own results (Tables II and III) would give exactly the same conversion factor as Mr. Edwards's equation. In other words, alkalimetric titration of this type could not decide the question of base exchange, though an alkalimetric titration of the Neumann type (where the ammonium ions are replaced by sodium and the ammonia boiled off) could do so.

Regarding Hundeshagen's equations, Mr. Thistlethwaite emphasised again that these could not be regarded as sacrosanct. Although the first one (without the nitric acid and water addenda) was based on full analysis by methods which Hundeshagen described in great detail, strangely enough he did not give any actual results of these analyses. It was really incredible that chemists ever since should have accepted this formula in the absence of the all-important experimental evidence. As to the second formula, the existence of the $2HNO_3$ was inferred solely from alkalimetric titrations, in which the number of equivalents of NaOH used varied from 23·14 to 23·46 for the heat-dried compound and from 25·28 to 25·43 for the desiccator-dried compound. Surely no chemist to-day would accept figures of such low precision and so far from whole numbers as definite proof of any formula.

Notes

THE ZINC URATE OXIDATION REACTION: A SELECTIVE TEST FOR ZINC

MANY samples of normal urine, when made slightly alkaline and oxidised by iodine, develop a red-purple colour that is deepened and stabilised by zinc. The chromogen has been found to be associated with the urochrome complex (Godfried[1]). In the course of a study of this reaction, the existence of a second chromogen that developed a blue colour was observed. It proved to be uric acid, and the reaction to which it gave rise was found to provide a delicate and selective test for zinc.

TEST CONDITIONS—About 20 mg. of finely-powdered uric acid are suspended in 4 ml. of a dilute (0·1 to 0·2 per cent.) zinc solution, and made moderately alkaline by addition of 2 to 4 drops (not more) of 20 per cent. sodium hydroxide solution. The mixture is well shaken, and then oxidised by gradual addition of 2 per cent. iodine in iodide solution, until the colour of the halogen just persists. Bromine water or hypochlorite is equally effective. The presence of $Zn^{..}$ is shown by the appearance of a pure blue colour, which remains when sufficient of the oxidiser has been added.

The test is tricky. It will not work in alkaline solutions at *p*H values below 12, and its delicacy is impaired by excess of strong hydroxides, which immobilise the metal as a zincate. When the unoxidised uric acid is in excess the colour fades, but can be restored by addition of more oxidiser, to which it is remarkably stable. The test will show $Zn^{..}$ in concentrations down to about 1 in 10,000. Below this, the pale yellow of the iodine is apt to obscure the reaction, and hypochlorite is then preferable as an oxidiser.

In concentrated solutions, the pigment separates out along with the zinc hydroxide. It is insoluble in all the common organic solvents, is reversibly bleached by mild reducing agents (including uric acid in alkaline solution) and is irreversibly discharged by weak acids. The test is of interest in being selective for uric acid and for zinc; no other substances have as yet been found capable of replacing either of the reactants.

EARLY HISTORY—In 1908, Ganassini[2] observed that when a strongly alkaline solution of uric acid is saturated with potassium persulphate and treated with a slight excess of 10 per cent. zinc sulphate solution until the precipitate no longer redissolves, it gradually acquires a blue-green colour. In this form, the test will reveal uric acid down to 0·01 per cent., and may be applied to urine by filtering off the pigmented zinc precipitate. Exposure to bromine, chlorine or sunlight hastens colour development on the moist precipitate. Vitali[3] reported that sodium peroxide was the only oxidiser capable of replacing the persulphate, and that zinc was the only metal that gave a blue pigment, although colours ranging from green to violet were given by $Cu^{..}$, $Co^{..}$, and $Ni^{..}$.

MECHANISM—The component of the pigment provided by the uric acid is elusive. Ganassini obtained negative results with all the other common purines, and some other nitrogenous compounds, namely: adenine, guanine, xanthine, hypoxanthine, heteroxanthine, paraxanthine, 3-methyl xanthine, 1:7-dimethyl xanthine, caffeine, theobromine, theophylline, nuclein, nucleic acid, alloxan, alloxanthin, allantoin, creatine, creatinine, glycine, tyrosine, leucine, hippuric acid and urea.

The form of the reaction with iodine as oxidiser was found to be negative with these compounds (excluding the substituted xanthines, which were not available), and with the recognised products of uric acid oxidation and related compounds, namely: 4:5-dihydroxy-4:5-dihydro-uric acid (uric acid glycol), dialuric acid, carbonyl diurea, cyanuric acid, cyamelide, allantoxanic acid, allantoic acid, allophanic acid, parabanic acid, violuric acid, barbituric acid, uracil, 5-amino uracil, biuret, arginine, guanidine, histidine, cyanate and carbamate. The uric oxidation derivatives were prepared according to the methods of Venables and Moore,[4] and Biltz and Klemm.[5] Application of the test at various stages during the oxidation of uric acid by liver uricase failed to show any production of the blue pigment, in absence of an external oxidiser. When adsorbed on the zinc precipitate, and washed free from contaminants, the pigment is stable indefinitely to light and air, and in some respects resembles a metallo-porphyrin type of compound. When it is decomposed by acid, urea is the only nitrogenous product so far identified. This suggested that the pigment might be a zinc allophanate, but allophanate prepared by mild alkaline hydrolysis of the ethyl ester failed to give any colour with zinc, under a variety of conditions. Incidentally, of all the compounds tested, only two reacted chromatically, namely dialuric acid and uric acid glycol, both of which in neutral or alkaline solution gave with zinc a yellow colour that, unlike the blue pigment, was reversibly bleached by acidification.

In its present form, the test is selective for zinc. No colour reactions were obtained with uric acid and any of the other common metallic cations, including: Al, Ba, Be, Ca, Cd, Ce, Fe, Hg, La, Li, Mg, Pb, Se, Sn, Sr, Ti, U and Zr. Some samples of cadmium gave a slight blue reaction, but this was traced to contamination with zinc. The colour reactions recorded by Vitali for Cu, Co, and Ni belong to a different category in that they are given by alkaline urate directly, without the need for an added oxidiser, and they are reversibly discharged by acidification. Silver and manganese also undergo characteristic colour changes, involving reduction, when added to alkaline urate.

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DEPARTMENT OF BIOCHEMISTRY
TRINITY COLLEGE, DUBLIN

W. R. FEARON
May, 1947

A MICRO FILTER THIMBLE

ALTHOUGH paper is the most widely employed filtering material,[1] its use in the form of a thimble appears to have been suggested only by Mack and Hecht,[2] who describe the method of preparing a small thimble by folding ordinary filter paper. In view of the difficulty of folding a paper in this way, we have designed a micro thimble which may be manufactured as a seamless cylinder of Whatman paper closed at one end as shown in Fig. 1.* The thimble is 10 mm. long and 5 mm. in diameter and weighs about 0·02 g.

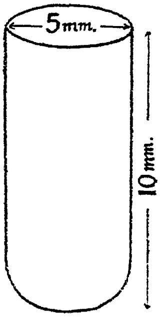

Fig. 1.

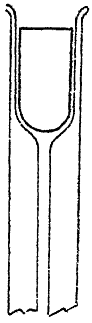

Fig. 2.

The thimble may be employed to filter small amounts of liquid by inserting it in a cylindrical funnel as shown in Fig. 2.

For the filtration of solutions by suction, the thimble is fitted over the end of quill or capillary tubing so that the arrangement can be used as an immersion filter or "filter-stick." To avoid tearing of the paper, the end of the glass tube should be "fire-polished," and the thimble worked on with a rotary motion. The curved bottom of the thimble affords additional strength, and solutions may be filtered very rapidly without damaging the paper. Provided the dry thimble fits tightly on the glass, the slight swelling which occurs in water ensures that there is no passage of the solid particles between the glass and the paper. The thimble is easily removed from the end of the tube and can be used several times.

A micro thimble of this kind has many other applications in microchemical, biological and general laboratory work.

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UNIVERSITY COLLEGE
CARDIFF

S. T. BOWDEN
February, 1946

THE FORMATION OF RACEMIC CALCIUM TARTRATE IN WINES

IN the course of studies on the formation of crystalline deposits in wine, it was observed that when old wines were being examined the crystalline deposit was usually composed almost entirely of calcium tartrate, and that this salt was not dextrorotatory as is the rest of the tartaric acid content of wines, but was optically inactive racemic calcium tartrate.

Various anomalies have been observed in the past, and the laws governing the deposition of crystallisable substances from solution, when applied to wines, seemed not to hold; these anomalies were ascribed to the phenomena of "seeding" and supersaturation. When it is appreciated that the dextrorotatory tartaric acid of the wine gradually changes to the optically inactive compound and that the solubility of the calcium salt of this acid is very low, the anomalies are rationally explained.

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* Made to the above design by arrangement with Messrs. H. Reeve, Angel & Co., Ltd., 9, Bridewell Place, London, E.C.4.

The solubilities of the various isomers are given as:

Dextro calcium tartrate	..	0·023 g. per 100 ml.
Laevo ,, ,,	..	0·025 ,, ,, ,, ,,
Racemic ,, ,,	..	0·003 ,, ,, ,, ,,

The differences between the solubilities of the various optical isomers of potassium hydrogen tartrate are not sufficiently large to have any effect on the production of crystalline deposits; although this substance is usually of importance in this connection, it behaves normally.

The typical crystalline form of racemic calcium tartrate can be reproduced by adding racemic tartaric acid to a wine, and the crystals then formed resemble those found naturally in the older wines.

The racemic salts can be easily made by heating the dextrorotatory salts with excess of potassium hydroxide in an oven at 150° C. for 2 hours.

The rate of formation of racemic tartaric acid in wines is dependent in the majority of cases almost exclusively on the temperature at which the wine is stored and the amount of tartrate radical present. The salts of racemic tartaric acid are less soluble at low temperatures, and their concentration can be reduced by refrigeration. If the wine is cooled to 0° C., as is normal practice for wineries using refrigeration methods, the concentration of racemic calcium tartrate is reduced by precipitation. If the wine is subsequently stored at a low temperature the rate of formation will naturally be small, and if it is stored at normal temperatures the time taken to produce enough of the optically inactive acid to raise the concentration of the calcium salt higher than the limit of its solubility, is between 6 and 9 months.

Summary—The natural dextrorotatory tartaric acid of wines undergoes auto-racemisation to produce the optically inactive acid, whose calcium salt is sparingly soluble and forms crystalline deposits.

Certain solubilities are given and the practical implications discussed.

THE LABORATORIES
VINE PRODUCTS LIMITED
KINGSTON ON THAMES

A. CAMBITZI
April, 1947

Official Appointments

OFFICIAL AGRICULTURAL ANALYST APPOINTMENTS

NOTIFICATION of the following appointments has been received from the Ministry of Agriculture and Fisheries since the last record in THE ANALYST (1947, **72**, 397).

Official Agricultural Analyst		*Appointments*
HERON, Neil (Deputy)	..	County Boroughs of Birkenhead, Blackburn, Bootle and Southport.

Ministry of Food

STATUTORY RULES AND ORDERS*

1947—No. 1317. The Canned Fruit and Vegetables (Amendment No. 2) Order, 1947. Dated June 26th, 1947. Price 2d.

This Order, which came into force on June 29th, 1947—

- (*a*) *removes apples, cherries, beetroot, carrots and macedoine of vegetables from the licensing provisions of the Canned Fruit and Vegetables* (*No.* 2) *Order,* 1946.
- (*b*) *prescribes maximum weights per can for sweet cherries, blackcurrants, redcurrants, strawberries and whitecurrants, and for cooked macaroni or spaghetti in meat sauce;*
- (*c*) *increases the strength of syrup prescribed for canned fruit* (*except apples and fruit packed in "A*10*" cans*) *by* 10 *degrees Brix, viz. from* 32·5° *maximum and* 29° *minimum to* 42·5° *maximum and* 39° *minimum;*
- (*d*) *prescribes new maximum prices for imported canned fruits; and*
- (*e*) *removes all canned vegetables except tomatoes from price control.*

— No. 2001. The Labelling of Food (Amendment No. 2) Order, 1947. Dated September 15th, 1947. Price 1d.

In respect of butter this Order, as from October 1*st,* 1947, *permits a claim on a label and in an advertisement that butter is a natural source of vitamin A, without requiring the quantity present to be specified.*

* Italics signify changed wording.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Modified Scharer Phosphatase Test for Milk and Soft Cheeses. W. Horwitz (*J. Assoc. Off. Agric. Chem.*, 1946, **29**, 129–165)—Scharer has developed two rapid methods, one for field use and the other for laboratory use. Disodium phenyl phosphate in a borate buffer is used as substrate and the very sensitive reagent of Gibbs (*J. Biol. Chem.*, 1927, **72**, 649; ANALYST, 1927 **52**, 360), *viz.*, 2 : 6-dibromoquinonechloroimide (BQC), is used to react with the liberated phenol to form a blue indophenol colour, the intensity of which is compared with that of a series of permanent inorganic standards. In the field test, the incubation period is shorter and the indophenol is extracted with butyl alcohol. Sanders and Sager (*J. Assoc. Off. Agric. Chem.*, 1945, **28**, 656; ANALYST, 1946, **71**, 82, Erratum p. 241) have developed a modified Scharer test designed for Cheddar cheese, the sodium borate buffer at *p*H 9·6 being replaced by a barium borate buffer of higher *p*H to allow for the buffer capacity of the sample, so that the *p*H of the incubated medium is about 9·6. A dilution method is introduced to determine the results of strongly positive tests, and the indophenol is extracted with butyl alcohol, the intensity of the colour being measured photometrically or against standards prepared from pure phenol.

Before the work of Sanders and Sager (*loc. cit.*) was available, trial tests with partly pasteurised milk had shown that Scharer's method was not entirely satisfactory, and *p*H measurements indicated that the procedure specified did not produce the optimum conditions under which the test should be conducted. A study of these conditions led to a modification of the Scharer laboratory method, the incubation *p*H being altered to 9·9 and the colour development *p*H to 9·5. The substrate concentration was increased and basic lead acetate solution (as originally used by Scharer) served as the protein precipitant. These modifications produce twice as much phenol from a given sample as that produced in the present Scharer procedure. By diluting raw products with pasteurised milk before incubation, accurate values for the total phosphatase content of raw milk and cream may be obtained. The proposed modification has been applied to soft cheeses. Since a severe loss of phosphatase occurs during the manufacture of cottage cheese, this product, if prepared from properly pasteurised skim milk, should show complete absence of phosphatase activity.

The following reagents are required. *Stock borate buffer*—Dissolve 28·43 g. of sodium pyroborate, $Na_2B_4O_7.10H_2O$, in about 800 ml. of water, add 125 ml. of *N* sodium hydroxide, and dilute to 1 litre. A 200-ml. portion of this stock borate buffer mixed with 125 ml. of *N* sodium hydroxide and diluted to 1 litre produces the *sample preparation buffer*, and a 15-ml. portion of the stock borate buffer diluted to 100 ml. provides the *colour development buffer*. *Standard curve buffer*—Dissolve 2·84 g. of the crystalline borax in 80 ml. of water, add 6 ml. of *N* sodium hydroxide, and dilute to 100 ml. A 10-fold dilution of this provides the *colour dilution buffer*. To prepare the BQC reagent dissolve 20 mg. of powdered 2 : 6-dibromoquinonechlorimide in 5 ml. of absolute ethyl or methyl alcohol, and store the solution in a vessel with a non-phenolic stopper in the freezing compartment of a refrigerator, discarding it after a week or if it develops a brown colour. *Buffer substrate* (0·005 *M*)—Dissolve 1·09 g. of crystalline disodium phenyl phosphate (phenol-free) in 5 ml. of water in a test tube, add 0·5 ml. of standard curve buffer and 0·04 ml. of BQC reagent, mix, and allow to stand for 15 min. Add 3 ml. of *n*-butyl alcohol, shake vigorously, centrifuge, and remove the upper layer. If any blue colour remains in the lower layer, repeat the procedure. Transfer the aqueous layer quantitatively to a litre flask, add 200 ml. of stock borate buffer, and dilute to the mark. The substrate has a *p*H of about 10·05, and is stable in the refrigerator for at least two weeks. The *n*-butyl alcohol (b.p., 116° to 118° C.) should be treated with 0·1 *N* sodium hydroxide until a small portion, when tested with bromothymol blue, gives a green or light blue colour. *Basic lead acetate solution*—Boil 250 g. of basic lead acetate with 500 ml. of water for 10 min., stopper, cool, filter, and dilute the filtrate to 500 ml. Store the bulk and withdraw small portions for daily use. *Phenol standards*—Dissolve 1 g. of phenol in 1 litre of 0·1 *N* hydrochloric acid and, if necessary, standardise the solution by the U.S.P. procedure. By successive dilution of a portion of this solution, prepare solutions containing 10 µg. (solution *a*), 2 µg. (solution *b*), and 0·5 µg. (solution *c*) of phenol per ml.

The standard curve—To 1 ml. of the standard curve buffer in each of a series of test tubes add the volumes of the working standard solutions to give concentrations of 0, 1·0, 2·5, 5·0, 7·5, 10, 15, 20, 25, 30, and 40 µg. of phenol when diluted to 10 ml., using solution *b* for concentrations up to 15 µg. and solution *a* for concentrations from 20 to 40 µg. per 10 ml. Finally, add the requisite amount of water to make 10 ml. The *p*H of these solutions is 9·5 to 9·6. Add 0·08 ml. of BQC reagent at appropriate intervals so that the colour of each solution may be read in 30 min. Mix immediately and allow to stand for 30 min. Read the colour intensity with a photo-electric colorimeter, using a filter with maximum transmittance near 610 mµ., or with the

Clifford photometer using filter 61 and the half-inch cell. Subtract the blank reading from the phenol scale readings and plot the differences on a large scale against µg. of phenol per 10 ml. If accurate values are desired for small amounts of phenol, use a butyl alcohol extraction. Prepare another series of phenol solutions containing 1 ml. of standard curve buffer and the amounts of standard phenol solution and water to yield concentrations of 0, 0·5, 1·0, 3·0, 5·0, 7·5, and 10·0 µg. of phenol per 10 ml., using solution *c* for concentrations up to 3·0 µg. per 10 ml. and solution *b* for the remainder, and finally adding the requisite amount of water to make 10 ml. Add 0·08 ml. of BQC reagent at appropriate intervals, mix, and allow to stand for 30 min. Add 5 or 10 ml. of *n*-butyl alcohol, shake vigorously, centrifuge, and draw off the upper layer with a pipette. Read the colour intensity of this layer with a photo-electric colorimeter using a filter with maximum transmittance near 650 mµ. or with the Clifford photometer using filter 65 and the half-inch cell. After subtracting the blank reading, plot the phenol scale readings as before.

Milk—To 1 ml. of milk in a Pyrex test tube add 10 ml. of the buffer substrate, mix well, and place in a water-bath at 37·5° ± 0·5° C. for 62 min. After incubation, place in boiling water for 3 to 5 min. To the thoroughly chilled liquid add 0·2 ml. of basic lead acetate solution from a pipette graduated to 0·01 ml. Mix immediately and vigorously, and filter through a rapid paper. The *p*H of the filtrate should be 8·7 to 9·0. To 5 ml. of the filtrate add 5 ml. of the colour development buffer, mix, add 0·08 ml. of BQC reagent, mix again, and allow to stand for 30 min. If necessary, adjust the *p*H of this liquid to 9·4 to 9·6. Measure the intensity of the colour at 610 mµ. If, during the colour development, it becomes apparent that the amount of phenol produced will exceed 25 µg., dilute 5 ml. of the liquid with 5 ml. of the colour dilution buffer, add 0·04 ml. of BQC reagent, mix well, and allow to stand for a further 30 min. Treat a blank determination similarly. Repeat the dilution as many times as may be necessary. For accurate results on raw products, repeat the determination, diluting the original sample with pasteurised milk (heated to 80° C. and cooled) so that the resulting colour will fall within the 5- to 20-µg. range without dilution.

If more accurate results are required with milks in which the amounts of phenol liberated are less than 5 mg., add, after the colour development, 5 or 10 ml. of *n*-butyl alcohol, shake, centrifuge, and measure the intensity of the blue-green colour in the alcohol at about 650 mµ. Conduct at least two blank determinations, using thoroughly pasteurised milk with each series, so that one may be available for dilution if necessary. The same blank determination may be used for a reading in aqueous solution and for a subsequent *n*-butyl alcohol extraction. Subtract the appropriate blank reading and transform the result into µg. of phenol from the standard curve. The concentration of phenol (µg./ml.) is given by µg. in the aliquot $\times \frac{11{\cdot}2}{5} \times$ dilution factor (2, 4, 8, etc., according as the solution is diluted once, twice, or thrice, etc.).

Soft curd cheese—For cream cheese of high *p*H (about 5·5), weigh 0·5 ± 0·02 g. in a Pyrex tube, add 0·5 ml. of a 1 + 4 dilution of the stock borate buffer, and form a smooth suspension. With cottage cheese or creamed cottage cheese, blend 50 g. with 50 ml. of the sample preparation buffer in the small container of a Waring blendor. Homogenise the product in a hand homogeniser, rinse the blendor with the homogenised material and re-homogenise. The *p*H of the prepared sample should be from 6 to 8·5. Weigh 1·0 ± 0·03 g. (≡ 0·5 g. of cheese) in a Pyrex test tube. Add 10 ml. of the buffer substrate, mix and, if necessary, adjust the *p*H to 9·9 ± 0·15 at 25° C. Proceed as directed for milk, but use 0·3 ml. of basic lead acetate solution. Adjust the *p*H of colour development to 9·4 to 9·6, if necessary. Samples showing high activity may be diluted, and those with low activity may be extracted as with milk. Conduct at least two blank determinations with each series, using prepared and weighed samples, which are immersed in boiling water for 1 min. and cooled before the substrate is added. Subtract the appropriate blank reading from the sample reading and calculate the results to µg. of phenol per g. of cheese. This is given by multiplying the number of µg. in the aliquot by 11·3/5 and by the dilution factor, and dividing by the weight of cheese in grams.

Readings in blank determinations may attain the equivalent of 0·6 µg. of phenol in the aliquot. Readings below 1·5 µg. per ml. with milk or 3 µg. per g. with cheese are therefore considered negative.

A. O. J.

Indole in Shrimp. R. E. Duggan and L. W. Strasburger (*J. Assoc. Off. Agric. Chem.*, 1946, **29**, 177–188)—From work of which little has been published it has been concluded that the indole content of shrimps could serve as an index of the extent of their decomposition. Two principal types of decomposition of raw shrimps have been reported. One is the putrefactive type, occurring when the shrimps, before icing, are exposed for a time and to a temperature favourable for bacterial incubation, and this type, once started, proceeds rapidly even though the shrimps are subsequently well iced. The other type is the ammoniacal type occurring in shrimps that have been held under well-iced conditions until decomposed; this is necessarily slow and is characterised by an odour of free ammonia and other odours associated with protein decomposition. To investigate the relationship between the degree of decomposition and the indole content, shrimps were allowed to decompose under conditions simulating commercial handling practices. The method adapted to the determination of indole was that of Clarke *et al.* (*Ibid.*, 1937, **20**, 475; ANALYST, 1937, **62**, 806).

Procedure—Pass the raw or cooked shrimps through a food grinder three times, and blend a 25- or 50-g. portion with 80 ml. of alcohol in a Waring blendor for several minutes. Using alcohol to rinse the mixing chamber, transfer the mixture quantitatively to a distillation apparatus consisting of a 500-ml. flask connected through a spray trap to a straight-bore condenser. The apparatus should be all-glass with the minimum amount of rubber tubing, but rubber stoppers covered with tin foil may be used if all-glass joints are not available. Distil slowly in steam until the alcohol has distilled off and the frothing stage of the distillation has passed. After 100 to 150 ml. have been collected, the rate of distillation may be increased. Adjust the heating so as to maintain the volume in the flask at 80 to 90 ml., and collect about 450 ml. of distillate in about 45 min. Rinse the condenser with alcohol and allow it to drain into the distillate.

Transfer the distillate to a 500-ml. separating funnel, add 5 ml. of diluted hydrochloric acid (1 in 20) and 5 ml. of saturated sodium sulphate solution. Extract successively with 25-, 20-, and 15-ml. portions of chloroform, shaking vigorously each time for at least 1 min. Combine and wash the 25-ml. and 20-ml. extracts in a 500-ml. separating funnel with 400 ml. of water, 5 ml. of the sodium sulphate solution, and 5 ml. of the diluted hydrochloric acid, and retain the wash water. Filter the combined extracts through a cotton-wool plug into a dry, 125-ml. separating funnel. Wash the 15-ml. extract, using the same wash water as before, and combine it with the other portions in the separating funnel. Add 10 ml. of the colour reagent (*infra*) to the combined extracts, shake vigorously for exactly 2 min., allow the acid layer to separate completely, and drain it into a dry, 50-ml., glass-stoppered graduated cylinder. Repeat the standing and draining until no more acid separates. Fill the cylinder to the 50-ml. mark with acetic acid, mix well, transfer the solution to a suitable photometer cell, and measure the colour photometrically at 560 mμ. The colour solution may be diluted with acetic acid provided that blank determinations are made at the same dilutions. Prepare a standard curve by this procedure by distilling alcohol containing a series of known amounts of indole. Determine the distillation blank in a like manner, omitting the addition of indole.

To prepare the colour reagent, dissolve 0·4 g. of *p*-dimethylaminobenzaldehyde in 5 ml. of acetic acid and add 92 ml. of phosphoric acid and 3 ml. of concentrated hydrochloric acid. Purify the reagent (*J. Assoc. Off. Agric. Chem.*, 1937, **20**, 477), as the purity exerts a strong effect on the intensity of the reagent blank. If the acetic acid used gives a colour with the colour reagent, it must be purified by distilling 500 ml. in an all-glass still with 25 g. of potassium permanganate and 20 ml. of sulphuric acid. The distillation blank (or reagent blank) has been found to vary from 1 to 2 mm. with the same reagents. This is due to colour reagent drained from the final extraction and to the difficulty of measuring the colour at extremely low concentrations. Indole was therefore considered to be absent from samples giving readings within 2 mm. of the distillation blank.

The following practical details were established. No indole is obtained in the first 100 ml. of distillate when 100 ml. or more of alcohol are present. About 60 per cent. of the indole present is found in the first 100 ml. of distillate collected after the alcohol has distilled off. When not more than 150 ml. of alcohol is present in the distillation flask, 95 to 100 per cent. of the indole present is contained in the first 450 ml. of distillate. Although water may be substituted for alcohol and less total distillate is then required, the use of alcohol is recommended to prevent frothing.

Absolutely fresh shrimps contain no indole and indole is not produced by cooking. The indole content increases as decomposition advances and is therefore a measure of the extent of decomposition. The indole content does not alter appreciably with cooking, the loss of indole being compensated for by the loss in weight of the shrimps. Storage of raw and cooked shrimps at commercial holding temperatures for long periods has no appreciable effect on the indole content, and the shrimps can be carried through normal commercial packing procedures, including canning, without formation of indole. Small amounts of indole are normally found in the commercially packed product. In 27 random market samples of cooked, peeled shrimps, the indole content ranged from 0 to 4·3 μg. per 100 g. (average 1·5 μg. per 100 g.). Shrimps cooked in brine previously used to cook decomposed samples acquire part of the indole and odours of decomposition of the contaminated brine. A. O. J.

Identification of Sulphonamides. A. Witte (*Pharm. Weekblad*, 1947, **82**, 135–139)—A number of identification reactions are described.

Procedures—I—To 1 ml. of an acid solution add an equal volume of 1 per cent. picric acid solution. Sulphapyridine alone gives an immediate precipitate, becoming crystalline. A few others give crystals within 10 minutes. II—To the acid solution add a few drops of a 5 per cent. solution of phosphomolybdic acid in dilute nitric acid. Clear and slightly turbid solutions are considered as negative tests. The precipitate with sulphaguanidine becomes crystalline after 1 hour, the others remain amorphous. III—To about 50 mg. of the compound add 5 drops of 0·1 *N* sodium hydroxide solution, dilute to 2 ml., heat to the boiling point, make up to 15 ml. with water, and filter. Add 1 drop of 10 per cent. copper sulphate solution and observe the colour of the precipitate after 1 min. Slightly turbid liquids are considered as negative tests. IV—To 1 ml. of the acid solution add 1 ml. of 4 *N* sodium hydroxide solution, 1 drop of liquefied phenol, and 2 drops of a solution of bleaching powder. Sulphanilamide alone gives a blue colour. Yellow and brown colorations are neglected. The colour reaction of Chávez (*Quart. J. Pharmacy*, 1944, **17**, 336) with thymol, is unreliable, as the colours vary according to the conditions.

The following table gives the results obtained with various compounds in the above tests and, in addition, the melting points of the compounds extracted from tablets, and that of the sublimate obtained from them. This sublimate, obtained from pyrimidine derivatives, gives the reaction of Raybin (*Pharm. Weekblad*, 1946, **81**, 134).

Compound	M.p. ° C.	M.p. of sublimate ° C.	Test I	Test II	Test III	Test IV
Sulphanilamide ..	165° to 168°	—	+	—	—	Blue
Albucide	181° to 184°	—	—	—	—	—
Sulphapyridine ..	189° to 191°	—	+ +	Beige	Yellow-green	—
Sulphathiazole ..	201° to 202°	—	+	Light brown	Violet	—
Ultraseptyl ..	244° to 256°	—	—	Light brown	—	—
Sulphasuccidine ..	183° to 185°	—	—	—	—	—
Sulphadiazine ..	256° to 258°	125° to 127°	—	Yellow	Violet-brown	—
Percoccide.. ..	235° to 237°	154° to 157°	—	Yellow	Brown-violet	—
Sulphamethazine	199° to 200°	149° to 151°	—	Yellow	Emerald green	—
Sulphaguanidine	189·5° to 192°	—	+	Lemon-yellow	—	—

G. M.

Microscopical Identification of Butacaine Sulphate (Butyn) and Procaine Hydrochloride (Novocaine). G. L. Keenan (*J. Assoc. Off. Agric. Chem.*, 1946, **29**, 327–329)—Butacaine sulphate and procaine hydrochloride both produce characteristic crystalline precipitates with potassium iodide. With the pure substances the reaction may be carried out as follows. *Butacaine sulphate*—Dissolve about 0·5 mg. of butacaine sulphate in one drop of 50 per cent. alcohol on a microscope slide. Draw into one side of the drop a drop of a 5 per cent. aqueous potassium iodide solution. Spherulites of needles, frequently in sheaves and also as single crystals, appear immediately. Remove the excess of liquid with small pieces of filter paper and allow the crystals to dry. They are then in a suitable condition for examination with the polarising microscope. In ordinary light, the needles are colourless; with crossed nicols, the extinction is parallel and inclined, the needles being probably of the monoclinic system, but it is characteristic of many of the crystals that extinction is not sharp. In convergent polarised light with crossed nicols, no interference figures are apparent. The refractive indices are α (not common), 1·550; β (most common), 1·570; γ, 1·680; all ± 0·002. With needles showing parallel extinction, α is shown lengthwise and γ crosswise.

Procaine hydrochloride—Add about 0·5 mg. of procaine hydrochloride to a drop of water on a microscope slide and place in the solution several crystals of potassium iodide. At first, minute droplets are formed, but these are soon followed by plates, many of which are hexagonal or quadrilateral in outline. On keeping the preparation, plates of various habits occur. Remove the excess of liquid from the slide with filter paper after a sufficient number of crystals have formed, and allow the slide to dry at room temperature. In ordinary light, the crystals are mostly hexagonal plates. With crossed nicols and parallel polarised light, it is characteristic of the plates that extinction is not sharp. The elongated plates show parallel extinction and positive elongation. In convergent polarised light with crossed nicols, biaxial interference figures are very common, brilliant, and clear-cut, showing especially sections perpendicular to the acute bisectrix. The axial angle is readily viewed within the microscope field, 2E being 60 ± with positive optical sign. The refractive indices are α, 1·570; β, 1·570; $\gamma > 1{\cdot}734$; all ± 0·002. A. O. J.

Determination of Narceine in Narceine Sodium Salicylate by means of Reinecke's Salt. C. G. van Arkel and P. J. G. Kramer (*Pharm. Weekblad*, 1947, **82**, 161–166)—Dissolve 1 g. of the compound in 250 ml. of water, filter the solution, and take 25 ml. for the analysis. Add to the solution 0·1 *N* hydrochloric acid to bring the *p*H to 3·2, using a comparison buffer solution and bromophenol blue as indicator. This value is finally adjusted at a dilution of 50 ml. Add, with stirring, 5 ml. of a 4 per cent. solution of Reinecke's salt prepared by rubbing the salt down with water, allowing to stand with frequent shaking, and finally filtering. Allow the mixture to stand for 24 hr. in a cool place, collect the precipitate on a sintered filter, wash with small portions of water, and finally with 40 per cent. alcohol, and dry for 24 hr. in a vacuum. During the washing of the precipitate remove each quantity of liquid as completely as possible, and then stir up the precipitate on the filter with small portions of wash liquid. The composition of the precipitate is apparently $Cr(NH_2)_2(SCN)_4.C_{23}H_{27}NO_8$, and 1 g. corresponds to 583 mg. of narceine. G. M.

Chromatographic Analysis of Alkaloidal Salts. F. Reimers and K. R. Gottlieb (*Dansk Tidsskr. Farm.*, 1943, **17**, 54–71). **F. Reimers** (*Ibid.*, 1945, **19**, 167–173)—*Procedure*—Dissolve a quantity of the salt, corresponding to about 5 ml. of 0·1 *N* hydrochloric acid, in 5 ml. of 90 per cent. alcohol, and pass the solution through a column 22 cm. long and 1 cm. in diameter, containing 10 g. of a suitable alumina. Wash through with 25 ml. of 90 per cent. alcohol, and titrate the liquid with 0·1 *N* hydrochloric acid to a green colour, using bromophenol blue as indicator. Satisfactory results were obtained with hydrochlorides of procaine, emetine, cocaine, pilocarpine, scopolamine, ethylmorphine, eucodal, percaine, pantocaine, and physostigmine salicylate. Ephedrine and homatropine require 15 g. of a more strongly adsorbing alumina, and 35 ml. of solvent for washing through; codeine requires 15 g. of alumina and 35 ml. of alcohol for washing through; strychnine 10 ml. of 90 per cent. alcohol washed through with 20 ml.; quinine and quinidine must be titrated with bromocresol green as indicator. With eucodal, on account of the low solubility of the base, it is necessary to wash through with 35 ml. of alcohol. The method is unsuitable for hydrochlorides of narcotine, papaverine, amphetamine, and apomorphine, and for codeine phosphate, oxedrine tartrate, dicodide bitartrate, and hyoscyamine hydrobromide. G. M.

Biochemical

Estimation of Penicillin in Serum. Use of Glucose, Phenol Red, and Serum Water. A. Fleming and C. Smith (*Lancet*, 1947, **252**, 401–402)—The method is a modification of that described by Fielding (*Brit. Medical J.*, 1947, Jan. 25th, 136) using Hiss's serum water, prepared by boiling 1 volume of serum with 3 volumes of distilled water, a fermentable carbohydrate, and Andrade's indicator; the medium is colourless, but on fermentation with a microbic growth it becomes bright red and a heavy precipitate forms. The present method uses serum water containing 20 per cent. of serum and 2 per cent. of glucose. Phenol red is a more suitable indicator than Andrade's indicator; at *p*H 7·6 the colour is cherry red, but at *p*H 6·8 there is a change to bright yellow, so that a very small amount of acid production can be detected. A haemolytic streptococcus that had previously been used as a test organism (Fleming, *Lancet*, 1944, **247**, 620) was found to be suitable; the inoculum used was 5 cu. mm. of a 24-hour broth-culture to 1 ml. of the medium, but there is considerable latitude in this. Good results were obtained when soda-glass capillaries were used for the incubation, but hard-glass capillaries caused some change in the *p*H of the fluids towards the acid side, making the results difficult to read.

The medium finally adopted contained 2·00 ml. of serum (human, horse, sheep, or ox), 2·00 ml. of 10 per cent. glucose solution, 6·00 ml. of distilled water, and 0·25 ml. of saturated phenol red solution. The medium can be made up in bulk, steamed, and distributed in small volumes, or quantities of 5 to 10 ml. can be made and boiled for a minute or two over a Bunsen flame. For making small quantities it is convenient to keep 10 per cent. glucose solution in sealed, 1-ml. ampoules that have been autoclaved.

Procedure—The test is carried out as previously described (Fleming, *loc. cit.*). Inoculate a suitable volume of the medium with the test organism. Make serial dilutions of the serum to be tested (25-cu.mm. volumes) in the inoculated medium on the surface of a paraffined slide. Touch each drop with the end of a capillary tube, when the fluid runs into the tube, seal, place horizontally on a Plasticine-covered slide, and incubate. Alternatively, avoid sealing by running the fluid towards the centre of the capillary by tilting the tube; the liquid remains in position when the tube is placed horizontally on the Plasticine-covered slide. By this method, the strongest concentration of the test serum is 1 in 2. If it is desired to use almost undiluted serum, mix 25 cu.mm. of undiluted serum with 5 cu.mm. of 50 per cent. serum water containing 5 per cent. of glucose and sufficient phenol red, and inoculate with 5 times as many streptococci.

After incubation, the tubes in which the streptococci have grown are bright yellow and show a heavy precipitate. There is often an intermediate tube in which there has been a colour change, but sufficient acid has not been produced to precipitate the serum. The tubes in which growth has been prevented by the penicillin are red-violet. The end-point is sharp and the results are very easily read. The test can be carried out in small test tubes by altering the volumes. The results obtained by the micro- and macro-methods are the same. E. M. P.

Organic

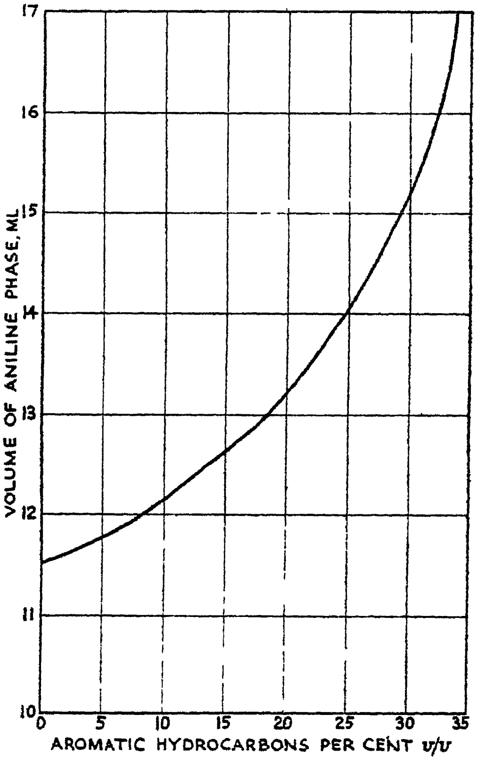
Determination of the Sugar Substitute—1-*n*-Propoxy-2-Amino-4-Nitrobenzene. F. Hoeke (*Chem. Weekblad*, 1947, **43**, 283–285)—This compound was used in Holland in place of saccharin. It is a yellow-orange, crystalline material with m.p. 49° C. The sweetening power is 4000 to 5000 times that of sugar and the anaesthetic action is 30 times that of cocaine. It was sold by the manufacturers diluted to a strength of 1 in 550, but in the retail trade, it was much weaker.

For its detection, a slightly acidified solution is made alkaline and shaken thoroughly with light petroleum (b.p. 40° to 50° C.). On evaporation of the latter, the residue is tested for the compound by its sweet taste, anaesthetic action, and melting point. The still alkaline, aqueous liquid is shaken out with ether to extract any dulcin, which is tested for; the remaining aqueous liquor is acidified and shaken out with ether, and the ethereal solution tested for saccharin. For the determination of 1-*n*-propoxy-2-amino-4-nitrobenzene, the method is varied according to the nature of the sample.

Procedure—Fat absent; dulcin and saccharin absent—Dissolve about 5 g. of the substance in 50 ml. of acidified water, and make alkaline with sodium hydroxide, whereupon the sweetening agent may separate out. Shake out the mixture three times with ether, wash with three portions of water, and evaporate the ethereal solution at 45° C. in a current of dry air to constant weight. *Fat absent; dulcin present*—Mix 5 g. with 5 g. of ignited sand, and extract in a continuous extractor with light petroleum (b.p. 40° to 50° C.). A period of 18 hours' extraction may be required. Distil off the light petroleum, dry in a current of air, weigh, and check the melting point. To determine the dulcin, the extraction thimble is allowed to dry, and the extraction continued with ether for 6 hours. The residue from the evaporation of this ethereal solution is the dulcin. *Fat absent; saccharin present*—Transfer about 5 g. to a separating funnel with the aid of 50 ml. of water. Add methyl orange, make alkaline, and shake out with three, 50-ml. portions of ether. Wash with three, 5-ml. portions of water, and recover the 1-*n*-propoxy-2-amino-4-nitrobenzene as before. Acidify the aqueous liquid with phosphoric acid and shake out with ether in a similar manner. The residue from the ether is the saccharin. *Fat absent; dulcin and saccharin present*—From an aqueous and slightly alkaline solution remove the 1-*n*-propoxy-2-amino-4-nitrobenzene by vigorous shaking with light petroleum (b.p. 40° to 50° C.). Extract the dulcin by shaking the still alkaline solution with ether. Acidify and shake out the saccharin into ether. *Fat present*—Extract the sample with ether in a continuous extractor, and remove the ether as before. Saponify the fat by boiling for 1 hour with alcoholic potash. Transfer the solution into a porcelain basin with a ground edge (this is essential), acidify with dilute hydrochloric acid, and evaporate off the alcohol, without evaporating to dryness. Filter warm through a wet filter paper, washing the residue and the filter with five portions of acidified water. If a little fatty acid passes through the paper, it does not interfere with the determination. Collect the acid filtrates in a separating funnel, make alkaline, and shake out the 1-*n*-propoxy-2-amino-4-nitrobenzene into ether as before. G. M.

Phase-volumetric Analysis of Mixtures of Benzine and Aromatic Hydrocarbons. H. J. A. de Goeij (*Chem. Weekblad*, 1947, **43**, 331–332)—This is a rapid method specially devised for the determination of aromatic hydrocarbons in solvent recovered from rotogravure processes.

Procedure—Place 10 ml. of the hydrocarbon mixture and 10 ml. of aniline in a stoppered measuring cylinder, graduated in 0·1 ml. Shake, adjust the temperature to 20° C., and read the volume of the aniline phase. If the content of aromatics is greater than 30 per cent. complete mixing occurs, and a definite quantity of light petroleum must be added before carrying out the test. Results are influenced to a small extent by the nature of the petroleum and aromatic fractions, and for the standardisation curve an average value is taken. This curve is given below. G. M.



Volumetric Determination of Organic Lead Compounds. F. Hein, A. Klein, and H. J. Mesée (*Z. anal. Chem.*, 1938, **115**, 177–183)—When polyalkyl lead compounds are titrated iodimetrically, the following reactions occur,

$$R_4Pb + I_2 \longrightarrow R_3PbI + RI$$
$$R_3PbI + I_2 \longrightarrow R_2PbI_2 + RI$$

Sometimes the di-iodocompound spontaneously forms PbI_2 and $R - R$, but other di-iodocompounds may be further iodinated according to the scheme

$$R_2PbI_2 + I_2 \longrightarrow PbI_2 + 2RI$$

To permit volumetric determinations of these compounds, the process must be stopped at a known stage by controlled conditions.

Lead tetra-ethyl—Ethyl alcohol solutions are stable for some months, as shown by titration with alcoholic iodine solution which, at room temperature, produces only first-stage iodination. If the solution is warmed to between 50° and 60° C., or if an excess of iodine be added, the excess being determined with standard thiosulphate, secondary reactions spoil the determination. The titration can be accelerated by adding 50 per cent. by volume of chloroform, glacial acetic acid, or methanol, none of which produces reaction beyond the first stage. The back-titration method in presence of methyl alcohol reduces the time of titration to 20 min., but sacrifices accuracy. Lead tetra-ethyl is less soluble in methanol than in ethanol, but when methanol is the solvent, titration with iodine-potassium iodide solution precipitates tri-ethyl lead iodide quantitatively in 10 to 15 min.

Dissolution of lead tetra-ethyl in ethylbenzene and methanol, treatment with an excess of iodine also dissolved in ethylbenzene, and back-titration with aqueous thiosulphate, starch being used as indicator, take 15 min. Direct titration with iodine is also accurate in these conditions, and takes 0·5 hr.

Lead tri-ethyl—A methanol solution is more stable than one in ethanol, although lead tri-ethyl is more soluble in the latter. Ethyl alcohol solutions of iodine are unsuitable either for direct titration or for the back-titration method. Titration with methanol-iodine solution until the reaction $2(C_2H_5)_3Pb + I_2 = 2(C_2H_5)_3PbI$ is complete must be employed, but detection of the end-point (a stable yellow colour) is made difficult by the yellow colour of the lead tri-ethyl itself.

Lead tetra-phenyl—Treatment with iodine in benzene solution under normal conditions results in considerable precipitation of lead iodide. Irradiation of lead tetra-phenyl and iodine in benzene solution by a 300-watt lamp placed between 15 and 20 cm. from the vessel, the contents of which have been heated to boiling for 10 to 15 min., results in a brown turbidity, which settles as lead iodide, leaving a violet-coloured solution of iodine in benzene. This excess of iodine may be titrated with thiosulphate solution in the absence of starch. The precipitated lead iodide may be determined gravimetrically, as such, or by conversion to lead sulphate. If the solution is irradiated at room temperature, less iodine than is required by the equation $Pb(C_6H_5)_4 + 3I_2 \longrightarrow PbI_2 + 4C_6H_5I$ is consumed, and the titration figure agrees with the equation

$$Pb(C_6H_5)_4 + 2I_2 \longrightarrow PbI_2 + 2C_6H_5I + C_6H_5.C_6H_5.$$

This view is supported by the detection of the characteristic odour of diphenyl when a benzene solution of lead tetraphenyl and iodine is irradiated under these conditions. M. E. D.

6-Nitro-2-mercaptobenzothiazole ("Nitro-Captax") as a Reagent for the Identification of Alkyl Halides. H. B. Cutter and H. R. Golden (*J. Amer. Chem. Soc.*, 1947, **69**, 831–832)—Reaction with primary or secondary alkyl halides gives crystalline derivatives, which may be converted to the corresponding sulphones, also crystalline.

Preparation of the reagent—Dissolve 50 g. of benzothiazole disulphide ("Altax") in 135 ml. of concentrated sulphuric acid, stir well, and cool. Add slowly and with constant stirring a mixture of 25 ml. of fuming nitric acid and 30 ml. of concentrated sulphuric acid, not allowing the temperature to rise above 50° C. Stir for a further 30 min. at room temperature, and pour, with stirring, into 3 litres of ice-water. Filter, and suspend the water-washed product in 500 ml. of water; add 450 ml. of water containing 40 g. of anhydrous sodium sulphite and 20 g. of sodium hydroxide. Pour the deep red solution, after filtration, into 3 litres of boiling, 5 per cent. hydrochloric acid, and filter off the precipitated mercaptan. Dry as thoroughly as possible on the filter, complete the drying by distillation with toluene, and crystallise from glacial acetic acid, using ether as wash liquid. Increase the yield by using the saturated acetic acid mother liquor for further crystallisations.

Preparation of 6-nitrobenzothiazole alkyl sulphides—Add 0·005 g.-mol. of the halide to a solution of 1·06 g. of the reagent in 10 ml. of butyl carbitol and 2·5 ml. of 2 *N* sodium hydroxide. If the halide is a chloride, add also 0·80 g. of potassium iodide. Heat under refluxing conditions for 15 min. for straight-chain iodides, 30 min. for bromides, and 60 min. for chlorides; double the period for secondary halides. Cool to room temperature, pour into 50 ml. of ice-water, and stand the vessel in ice until the derivative crystallises. Filter, and wash with dilute sodium hydroxide solution and water until a clear filtrate is obtained. Dissolve the product in methanol and boil for 1 min. with Norit, then filter hot, and cool the filtrate. Tertiary halides give no derivatives.

Oxidation to sulphones—Dissolve 0·01 g.-mol. of the sulphide in glacial acetic acid, using 50 ml. for 1 g., and warming if necessary. Add 40 ml. of 7·5 per cent. potassium permanganate solution, and more acetic acid, if necessary to maintain solution. Add sulphurous acid to reduce the excess of permanganate and the manganese dioxide formed. Precipitate the sulphone by adding ice-water, and recrystallise the filtered product from aqueous ethyl alcohol.

Results—The following are melting points, in ° C., of some alkyl-6-nitrobenzothiazolyl sulphides, $RC_7H_3N_2S_2O_2$, and the corresponding sulphones, $RC_7H_3N_2S_2O_4$, where R is the alkyl substituent group.

R	$RC_7H_3N_2S_2O_2$	$RC_7H_3N_2S_2O_4$
Methyl	134°	186°
Ethyl	103° to 104°	160°
n-Propyl	94° to 95°	181°
i-Propyl	75° to 76°	154°
n-Butyl	64° to 65°	152°
i-Butyl	68° to 69°	124°
s-Butyl	oil	121°
n-Amyl	48° to 49°	125°
i-Amyl	56° to 57°	115°
n-Hexyl	55·5°	111·5°
n-Heptyl	39·5°	123·5°
Allyl	72° to 73°	no derivative
Benzyl	114° to 115°	194°
cyclo-Hexyl	100° to 101°	189°

M. E. D.

Inorganic

Quantitative Separation of Barium from Strontium. G. L. Beyer and W. Rieman, 3rd (*Anal. Chem.*, 1947, **19**, 35–37)—Using the method of double precipitation of barium chromate, increased accuracy is obtained by precipitating at room temperature at a *p*H value of 4·6, boiling to render the precipitate easily filterable, and digesting at room temperature to reduce co-precipitation of strontium chromate. Sodium reagents are used to reduce co-precipitation, and the optimum *p*H is obtained by careful control of reagents and wash liquid. The wash liquid contains 2·23 g. of sodium dichromate dihydrate, 9·63 g. of sodium acetate trihydrate, and 3·1 ml. of 12 *M* hydrochloric acid in 1 litre of water; this solution has *p*H 4·6 and approximates closely in composition to the mother liquors.

Procedure—Treat a sample containing between 0·4 and 1 mg.-mol. of barium chloride and between 0 and 10 mg.-mol. of strontium chloride with 3 ml. of 2·0 *M* hydrochloric acid. Dilute to 185 ml., add 5·0 ml. of 0·40 *M* sodium dichromate and, with continuous stirring, 10 ml. of 1·31 *M* sodium acetate, taking between 1·5 and 2·5 min. for the addition. Heat to vigorous boiling in 3 to 6 min., cool rapidly to room temperature, and digest for 1 hr. at room temperature. Decant the liquid through a sintered-glass crucible of medium pososity, and wash three times by decantation with 20-ml. portions of the buffered wash liquid. Transfer the bulk of the precipitate to the crucible by means of more wash liquid, and wash further until a total of between 150 and 200 ml. of solution has been used, and then with 30 ml. of very dilute sodium hydroxide solution (*p*H 9 to 10).

Return the bulk of the precipitate to the original beaker by means of 10 to 20 ml. of water, and add 3·0 ml. of 2·0 *M* hydrochloric acid. Boil, cool, and dilute to 185 ml.; add 3·75 (± 0·1) ml. of 0·40 *M* sodium dichromate, followed by 8·55 (± 0·1) ml. of 1·31 *M* sodium acetate slowly and with stirring, as before. Heat to boiling, cool rapidly, and digest at room temperature. Filter through the same crucible, transferring the precipitate quantitatively by means of the buffered wash solution. Wash with 150 to 200 ml. of the solution, and then with the dilute sodium hydroxide solution until free from chromate. Transfer most of the precipitate to a 500-ml. flask, drawing 50 ml. of 0·5 *M* hydrochloric acid through the crucible to complete the transfer, and rinse with water. Dilute the solution and determine the chromate volumetrically by the iodimetric method.

With a barium content between 0·4 and 0·01 mg.-mol., the solution should be allowed to stand overnight.

Results—In the presence of 0 to 10 mg.-mol. of strontium, 0·4 to 1·0 mg.-mol. of barium may be determined to ± 0·003 mg.-mol., but low results are obtained with less barium.

By allowing the solutions to stand overnight before filtration, results of similar accuracy were obtained with 0·4 to 0·02 mg.-mol. of barium, in presence of 10 of strontium. Positive errors are incurred if 0·5 to 1·0 mg.-mol. of barium is used, owing to contamination of the precipitate by strontium on prolonged standing.

In the presence of 1 mg.-mol. of barium, 1 mg.-mol. of cadmium, calcium, cobalt, copper, mercury, magnesium, manganese, nickel, or zinc has no effect on the accuracy of the method. Beryllium, and aluminium both to produce low results, due probably to co-precipitation of some of the barium as barium hydroxide.

In determining 1 mg.-mol. of barium, the presence of 5 mg.-mol. of sodium chloride has no effect on the accuracy of the results, but 5 mg.-mol. of sodium nitrate produce low results, probably owing to co-precipitation of barium nitrate. M. E. D.

Volumetric Determination of Mixtures of Ferrocyanide and Arsenious Acid. R. Lang (*Z. anal. Chem.*, 1938, **115**, 103–104)—The titration of mixtures of ferrous salts and arsenious acid by ceric sulphate (Glen, *Ibid.*, 1933, **95**, 305) is inapplicable since ferricyanide poisons the catalytic action of the osmic acid used.

Procedure—To 100 ml. of the solution, *N* with respect to sulphuric acid, add 2 drops of 0·01 *N* ferroin (*o*-phenanthroline - ferrous complex) solution, and titrate the ferrocyanide content with 0·1 *N* ceric sulphate until the colour changes from brownish to light green. Add 1 to 2 g. of sodium chloride, and 5 drops of 0·0025 *M* potassium iodate, when the brown colour of the solution should return. Titrate the arsenious acid then with

0·1 *N* permanganate to the return of the light green coloration.

On mixtures ranging from 4 parts of 0·1 *N* ferrocyanide and 1 of 0·1 *N* arsenious oxide, to 4 of arsenious oxide and 1 of ferrocyanide the maximum deviation was 1 part in 1000. The presence of chromium ions does not decrease the accuracy.

M. E. D.

Colorimetric Determination of Titanium with Disodium-1 : 2-dihydroxy-benzene-3 : 5-disulphonate. J. H. Yoe and A. R. Armstrong (*Anal. Chem.*, 1947, **19**, 100–102)—The reagent gives a stable yellow complex of constant colour intensity in the *p*H range 4·3 to 9·6. Interference by ferric iron is eliminated by dithionite reduction at *p*H 4·7. The transmittancy of the ferric complex can be measured at *p*H 4·7 with light of wavelength 560 mμ. without interference from the titanium. Sodium dithionite ($Na_2S_2O_4$) destroys the colour due to iron, and the titanium complex is measured at 410 mμ., as dilute dithionite solution absorbs below this wavelength. A large excess of reagent must be present to give conformity with Beer's law. The test is, spectrophotometrically, sensitive to 1 part in 2 × 10^8, but 0·01 to 0·08 p.p.m. is the most convenient range for comparison in 50-ml. Nessler cylinders, and differences of 0·03 p.p.m. can be detected between 0·1 and 0·4 p.p.m. of titanium. As a drop reaction on a white porcelain plate, 0·1 μg. of titanium in 0·05 ml. of solution can be detected.

The following ions do not interfere at the concentrations indicated in parts per million, tervalent arsenic 20, ortho-arsenate 20, pyroborate 100, orthoborate 200, beryllium 100, bromide 2500, cadmium 300, chloride 6000, iodide 1000, magnesium 100, manganese 250, nitrate 1250, monohydrogen phosphate 1000 (P_2O_5), zinc 500, succinate 1200, and biphthalate 0·05 *M*. The concentrations of acid radicals given apply to the main element.

The vanadyl radical forms a purple complex, whilst uranyl, molybdate, and osmate ions all give yellow complexes and hence must be absent. The iron complex is red in alkaline solution, and blue in acid solution, in which dithionite reduces it at *p*H values less than 5. The titanium coloration does not develop its maximum intensity below *p*H 4·3, so the solution must be buffered to between *p*H 4·3 and 5. Oxidising agents, including atmospheric oxygen, produce a return of the blue colour due to the iron complex.

Aluminium, calcium, ceric, mercuric, lead, stannic, zirconyl, and tungstate ions consume reagent and reduce the intensity of the titanium coloration. The tungsten complex absorbs strongly at 410 mμ., and interferes with spectrophotometric determinations of titanium.

Procedure—Remove interfering ions from a solution of iron and titanium, dilute in a calibrated flask, and take an aliquot portion containing 0·04 mg. of titanium. Transfer to a 50-ml. Nessler cylinder, add 5 ml. of 4 per cent. aqueous solution of the reagent and aqueous ammonia until the solution is neutral to Congo red. Add 5 ml. of a buffer solution containing equal volumes of *M* acetic acid and *M* sodium acetate solutions, dilute to 50 ml., and mix. Dissolve in the solution 25 mg. of sodium dithionite ($Na_2S_2O_4.2H_2O$) by gentle agitation, and compare within 15 min. with standard titanium solutions. The standards, being iron-free, have no added dithionite and are stable for several weeks.

Iron and titanium may be determined in the same sample by measuring the transmittancy of the iron complex at 560 mμ., and then that of the titanium complex at 410 mμ. If these contents are of different orders of magnitude, aliquot portions of suitable size must be taken. Spectrophotometric determination is applicable in solutions with up to 3 p.p.m. of titanium, and 10 of iron; iron may be determined visually by using filters to eliminate light of wavelength less than 530 mμ.

The method has been checked on Bureau of Standards' samples of argillaceous limestone, felspar, fluorspar, soda-lime glass, and glass sand, treated to ensure elimination of fluorine and complete extraction of iron and titanium. Data obtained by the new method are compared with the standard values.

M. E. D.

Polarography of Mercury Solutions. K. Poma (*Bull. Soc. Chim. Belg.*, 1945, **54**, 277–285)—The author has made a study of the polarography of mercury solutions, with sodium perchlorate as the indifferent electrolyte. To prevent possible adsorption at the mercury drop, tylose was added. In the presence of oxygen, metallic mercury dissolves appreciably in a solution of perchlorate, so that it was necessary to remove oxygen from the solution either by a current of hydrogen, or by addition of sodium sulphite, which could also be used as an indifferent electrolyte. *Solutions with free mercury ions*—Sulphite must not be added as it reduces the mercury to metal. In the presence of perchlorate, there is a wave for both mercurous and mercuric ions, the separation potential being + 0·36 v. for 0·001 *N* solutions. *Solutions with an excess of iodide*—Both mercuric and mercurous ions form the ion HgI_4'', mercury being liberated when mercurous ions are used. In both cases the reduction potential in 0·001 *N* solutions is − 0·305 v. in perchlorate solution, and − 0·310 in sulphite solution. The concentration may be determined from the height of the wave, but as this does not vary linearly with concentration, standardisation is necessary. *Solutions with an excess of cyanide*—With perchlorate, there is a normal wave at − 0·655 v. for 0·001 *N* solutions. The phenomena in sulphite solutions are more complicated. *Solutions with an excess of thiocyanate*

—Both mercuric and mercurous salts give the ion $Hg(SCN)_4''$, metallic mercury being liberated when mercurous salts are used. For 0·001 *N* solutions, the separation potential is 0·01 v. in perchlorate and − 0·90 v. in sulphite solutions. *Amperometric titration*—Polarographic titration of mercury with thiocyanate, or *vice versa*, may be carried out with an accuracy of 1 per cent. Both solutions are prepared in 0·1 *N* perchloric acid, and 1 per cent. of tylose is added. The titration curve is a straight line cutting the axis at the equivalence point. A similar titration with cyanide in place of thiocyanate is less satisfactory. In acid solution, cyanide ions give no wave, and the separation potential is dependent on the *p*H of the solution. A buffer solution containing 35·5 g. of ammonium chloride and 30 ml. of 13 *N* ammonia in 150 ml. is therefore used. The potential to which the titration is carried is that at which a pure solution of mercuric cyanide in the buffer solution gives no deflection on the galvanometer. This potential is apparently a function of the concentration of the mercuric cyanide. Although normal titration curves are obtained with known solutions by a suitable choice of potential, the titration is uncertain for unknown solutions. For the reverse titration, the acid mercury solution is placed in the cell and titrated with the cyanide. Since the mixture remains acid, the anodic wave for cyanide does not appear. Here, also, the required potential depends on the concentration, but it may be found by carrying out a titration at an arbitrary potential until there is no further deflection of the galvanometer and altering the potential, without further addition of reagent, until the galvanometer reading is zero. This is then the suitable potential for the titration of the unknown solution. For 0·001 *N* solutions titrated with cyanide, the error is of the order 10 per cent. At a concentration of 0·01 *N*, the accuracy is satisfactory. G. M.

Determination of Nickel in Cobalt Steels and Alloys. H. Kirtchik (*Anal. Chem.*, 1947, **19**, 95–96)—Existing methods are inadequate in that nickel and cobalt may not vary over wide limits; also, gravimetric determination of low nickel contents gives low results since the cobalt must be removed by organic solvents. The method now described involves the formation of cyanide complexes, and the selective oxidation of the cobalt complex by hydrogen peroxide. After removing the excess of peroxide, formaldehyde treatment converts the unchanged nickel complex to an ionisable form, and the nickel can be separated as the dimethylglyoxime complex. The method can be applied to electro-deposited cobalt-nickel mixtures. Aluminium, beryllium, niobium, chromium, copper, iron, manganese, molybdenum, silicon, tantalum, titanium, vanadium, tungsten, or zirconium can be present.

Procedure—Dissolve a sample of 0·03-g. nickel content in *aqua regia*. If the material is iron-based and more than 0·5 g. is taken for analysis, evaporate the solution to dryness. Add 20 ml. of concentrated hydrochloric acid and evaporate to a thick syrup. When cool, add 50 ml. of diluted hydrochloric acid (5 + 2) and transfer to a 300-ml. separating funnel, using between 25 and 50 ml. of this acid to rinse the beaker. Extract the iron by shaking with 150 ml. of *iso*propyl ether. Return the aqueous layer to the beaker. Add 25 ml. of the cold, diluted hydrochloric acid and 10 ml. of *iso*propyl ether to the funnel, and shake again. Combine the acid layers, concentrate them to a small volume, and add 25 ml. of nitric acid.

To the solution add 15 ml. of 70 per cent. perchloric acid, concentrate to fuming, and boil for 5 to 10 min. adding periodically 0·5-ml. portions of concentrated hydrochloric acid to expel the chromium. Cool, add 50 ml. of water, and boil off the free chlorine. If aliquot portions are to be taken, dilute the perchloric acid solution, before or after removal of the chromium, filter it into a graduated flask, wash with 2 per cent. hydrochloric acid solution and dilute to the mark when cool. Chromium may also be removed by treating the perchloric acid solution with 20 ml. of 20 per cent. sodium hydroxide solution and saturated bromine water, boiling for 4 min., filtering, and washing with hot, 0·5 per cent. sodium hydroxide solution and discarding the filtrate. Transfer the paper and precipitate to the original beaker and dissolve it in an excess of hot, diluted (4 + 1) nitric acid and a few drops of 3 per cent. hydrogen peroxide. Add 5 ml. of 70 per cent. perchloric acid and evaporate to fuming, cool, dilute to 50 ml. with water, and boil for 1 or 2 min.

Adjust the chromium-free solution obtained by one of the above methods to be weakly acidic by means of 50 per cent. sodium hydroxide solution and diluted hydrochloric acid, and add cautiously 0·5-g. portions of sodium cyanide until the precipitate first formed redissolves, although in the presence of niobium, tantalum or zirconium, a faint precipitate remains after the heavy, coloured one dissolves; this should be disregarded. Dilute to 200 ml. with warm water and add 15 per cent. hydrogen peroxide solution until the solution is honey-yellow in colour. Boil gently to destroy the excess of peroxide, and concentrate to 75 ml., adding more cyanide and peroxide if necessary to maintain the colour of the solution. Dilute to about 100 ml. and heat to 60° C. Add, with stirring, 0·5 to 0·75 g. of dimethylglyoxime, 5 to 10 ml. of 40 per cent. formaldehyde solution, and allow to stand for 1 to 3 hr., or overnight if the nickel content is less than 2 per cent. Filter the precipitate, and wash 10 to 15 times with cold water. Return the paper and precipitate to the original beaker, and destroy organic matter by treatment with 25 ml. of nitric acid and 10 ml. of 70 per cent. perchloric acid, and concentration to dense fuming. Cool, add 50 ml. of water, 5 to 10 ml. of 6 per cent. sulphurous acid, some paper pulp, and boil for, at least, 3 min. Digest at 75° C. until the supernatant liquid is clear, and filter into a 600-ml. beaker; wash the residue 10 or 12 times with hot, 1 per cent. hydrochloric acid solution. Determine the nickel in the solution gravimetrically, volumetrically, or by electrodeposition.

Gravimetric determination—Buffer the solution with tartaric acid, and add dimethylglyoxime and dilute aqueous ammonia to precipitate the nickel complex, which may be weighed as such or ignited to nickel oxide.

Volumetric determination—Treat the nickel solution with 10 ml. of 10 per cent. ammonium citrate solution, 10 ml. of diluted sulphuric acid (1 + 1), and a 3-ml. excess of diluted aqueous ammonia (1 + 1). Dilute to 150 ml., and cool to room temperature. Add 3 ml. of 10 per cent. potassium iodide solution, and from burettes 3 ml. of standard silver nitrate, and then standard potassium cyanide to discharge the opalescence. Add more standard silver nitrate carefully to the re-appearance of cloudiness; this is taken as the end-point.

The silver nitrate solution (5·7890 g. per litre) should be equivalent to 0·001 g. of nickel per ml. The cyanide solution should contain about 4·5 g. of potassium cyanide and 1 g. of potassium hydroxide per litre, but as it deteriorates on keeping it must be checked as it is used. Take 100 ml. of water, 2 or 3 drops of concentrated hydrochloric acid, 20 ml. of diluted sulphuric acid (1 + 1), and a 3-ml. excess of diluted aqueous ammonia (1 + 1), cool, add 3 ml. of 10 per cent. potassium iodide solution, and 15 to 20 ml. of standard silver nitrate solution from a burette, and titrate as above with the cyanide solution, finishing with 1 drop of silver nitrate.

M. E. D.

Agricultural

Determination of DDT in presence of DDD. H. V. Claborn (*J. Assoc. Off. Agric. Chem.*, 1946, **29**, 330–337)—The presence of DDD, 1:1-dichloro-2:2-bis(chlorophenyl)ethane, in commercial samples of DDT has been reported (Haller *et al.*, *J. Amer. Chem. Soc.*, 1945, **67**, 1591), but it occurs in such small amounts that, although it interferes with many of the methods for determination of DDT, it has hitherto been possible to ignore it. DDD is now available commercially as an insecticide and a method is needed to distinguish between these two closely related compounds. The method described by Stiff and Castillo (*Science*, 1945, **101**, 440; ANALYST, 1945, **70**, 350; *J. Biol. Chem.*, 1945, **159**, 545; *Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 316; ANALYST, 1946, **71**, 450) for the determination of DDT is specific for that insecticide even in presence of large amounts of DDD. These authors used a reagent consisting of xanthydrol in dry pyridine, but it is now found that when prepared with absolutely anhydrous pyridine the reagent will not react with DDT. The water content of the pyridine may range from 0·1 to 0·5 per cent., but it is necessary that it should be constant, and to accomplish this 0·2 per cent. of water is added to anhydrous pyridine. To ensure constancy of the intensity of the green colour of the reagent, it is matched against the colour of a solution of 5 mg. of dithizone in a litre of carbon tetrachloride. With such changes, the method is essentially that of Stiff and Castillo (*loc. cit.*).

Preparation of the standard curve—Prepare a standard solution of pure *p*:*p'*DDT in benzene, containing 100 μg. per ml. Measure into 8 test tubes 0·25, 0·50, 0·75, 1·00, 1·25, 1·50, 1·75, and 2·00 ml. of the standard solution and remove the solvent by immersing the tubes in a steam-bath and, finally, removing the last traces by means of a current of air. Set the tubes aside until the reagent has been prepared.

Weigh 100 mg. of xanthydrol into a 500-ml. standard-taper round-bottomed flask. Add 500 ml. of pyridine (*infra*), attach an air condenser, heat to moderate boiling, and add through the condenser the number of pellets of potassium hydroxide (which have not been unduly exposed to air) previously found to be equal to 2·0 to 2·2 g. Swirl the flask continuously or frequently while boiling gently. The solution will first acquire an orange colour, then a light green and, finally, a dark green colour. When the colour reaches the intensity of that of a solution of 5 mg. of dithizone in a litre of carbon tetrachloride, discontinue the heating. Pour the solution into a glass-stoppered flask leaving the potassium hydroxide in the reaction flask. When the solution is cold, the colour will change to yellow. Set the reagent aside for 15 min. before use. It is stable for 2 hr., but must be mixed before use.

Measure 5 ml. of the reagent into a clean, dry test tube and stopper tightly with a cork covered with tin-foil. Place the tube in an oil-bath at 120° ± 2° C. for 8 min., and then in cold water for 1 min., remove, mix, pour into a 1-cm. cell, and read immediately, using a filter transmitting at 510 mμ. Treat each of the tubes containing the DDT in the same manner as this blank tube, measuring the reagent into the tube immediately before placing it in the oil-bath. Subtract the blank reading from the observed readings and prepare the standard curve by plotting the readings against the concentrations in μg. The curve should be linear.

Determination in apple spray residues—Wash the apples by the method of Wichmann *et al.* (*J. Assoc. Off. Agric. Chem.*, 1946, **29**, 188). Measure 2 ml. of the benzene solution into a test tube, evaporate the solvent by immersion in a steam-bath, and remove the final traces by means of a current of air. Set the tubes aside, prepare the reagent, measure 5 ml. of the reagent into each tube, and determine the DDT in the same way as in the preparation of the standard curve.

To prepare pyridine for the reagent add 50 g. of potassium hydroxide pellets to 700 ml. of pyridine in an Erlenmeyer flask fitted with a reflux condenser (cork or rubber stoppers must not be used). Heat on the steam-bath for 1 hr., or until the potassium hydroxide has dissolved, and decant the cooled pyridine layer into another similar flask. Add 50 g. of potassium hydroxide and heat under reflux on a hot-plate for 45 min. Decant the pyridine and distil in an all-glass distillation apparatus. To the distillate add 0·2 per cent. by volume of water and store the mixture in a glass-stoppered bottle.

Qualitative test for DDT on apples—Place an apple on a funnel draining into a Pyrex test tube. Pour 5 ml. of pyridine containing 2 mg. of xanthydrol per ml. over the surface of apple including the stem and calyx depressions. Add 4 pellets of potassium hydroxide to the drainings, boil gently for 5 min., and cool for 1 min. A red colour indicates the presence of DDT. A. O. J.

Determination of Traces of Copper in Vegetable Material by Means of Diethyldithiocarbamate. J. F. Reith, J. T. L. B. Rameau, E. G. Mulder, L. Seekles, L. C. E. Kniphorst, and W. B. Deijs (*Chem. Weekblad*, 1947, **43**, 106–109)—This is a report of a Committee of the Organisatie voor Toegepast Natuurwetenshappelijk Onderzoek. After considering the two reagents available for the purpose, dithizone and "carbaminate" (sodium diethyldithiocarbamate), preference is given to the latter. Dithizone is more sensitive and selective, but requires the method of mixed colorimetry with measurement of the extinction coefficient at a definite wavelength. Carbon tetrachloride extracts from an alkaline carbaminate solution not, only copper, but also complexes with mercury$^{\text{II}}$ silver, lead, cadmium, and zinc—all colourless—and also nickel, cobalt, and bismuth, which give yellow solutions the colour intensity of which is not more than one twentieth that of copper. Disturbance by nickel and cobalt may be eliminated by addition of dimethylglyoxime. The effect of manganese is neutralised by addition of citrate, or by washing with aqueous ammonia. Citrate serves the further purpose of preventing precipitation of calcium phosphate.

Method—*Reagents* should be copper-free; *i.e.*, give a blank of not more than 2 μg. Glass vessels must be previously washed with nitric acid. Carbon tetrachloride is purified by shaking with concentrated sulphuric acid, washing with water, drying with solid sodium hydroxide, and distilling. Aqueous ammonia and nitric acid should be distilled. *Sodium citrate solution*: 37 g. of crystalline sodium citrate in water to 100 ml. The solution is shaken out with excess of dithizone solution in chloroform, washed with chloroform until the latter remains colourless, and freed from chloroform by evaporation.

Procedure—Place 1 to 5 g. of dried material (containing 20 to 100 μg. of copper), in a short-necked flask of 150- to 250-ml. capacity, and moisten with three times its weight of water. Add 5 ml. of concentrated sulphuric acid and 5 ml. of 65 per cent. nitric acid and heat, cautiously at first, until charring commences. Add a few drops of nitric acid, and heat again until the solution is black, and nitrous vapours are no longer present. Repeat the oxidation with nitric acid until the solution is colourless or very light yellow and does not change in colour after five minutes' boiling with the sulphuric acid. Remove nitrosylsulphuric acid by adding an equal volume of water and evaporating on the water-bath. Add to the solution sufficient citrate solution to keep the copper in solution (5 to 15 ml.), nearly neutralise with aqueous ammonia, and transfer to a separating funnel. Make alkaline to phenolphthalein paper with aqueous ammonia, add 5 ml. of 0·1 per cent. carbaminate solution and 15 ml. of carbon tetrachloride, and shake strongly for 1 min. Run off the lower layer into a 25-ml. measuring flask, and repeat the extraction with two further 5-ml. portions of carbon tetrachloride. Make up the volume of the carbon tetrachloride solution to 25 ml. The extraction should be done in diffused light since the copper compound is light-sensitive. To remove any iron or manganese, shake the solution vigorously with half its volume of 0·5 *M* ammonia. This operation may be omitted if the quality of citrate solution originally used is as high as 10 ml. per g. of original material. Filter the carbon tetrachloride solution through ash-free paper, and immediately determine the extinction at about 430 mμ. The photometer must be standardised against a suitable copper solution. Alternatively, compare the colour in a colorimeter against a known quantity of copper, prepared similarly. G. M.

Bacteriological

Determination of Traces of Iron in Bacteriological Media. A. Tasman and L. Smith (*Chem. Weekblad*, 1947, **43**, 248–253)—The presence of traces of iron is of great significance in the production of toxins and similar products, but there is some difficulty in its determination owing to the presence of phosphates in the media. By ashing, these are converted to pyrophosphates and all of the iron is not recovered.

Reagents—*Concentrated hydrochloric acid.* Distil in glass with addition of a little silver nitrate to hold back ferric chloride. *Distilled water*, chloride-free. *Ammonia*—25 to 26 per cent., distil in glass. *Hydrogen sulphide.* Wash the gas, prepared from ferrous sulphide and hydrochloric acid, with ammonium sulphide solution and water, and filter through glass wool. Do not use red rubber tubing that has been vulcanised with the addition of ferric oxide. *Acetate buffer.* Distil acetic acid over silver nitrate and dissolve 288 ml. with 100 g. of sodium hydroxide in water to make 1 litre. *Sodium hyposulphite* ($Na_2S_2O_4$) *solution*, 20 per cent., iron-free; purify by the method of Hill (*Proc. Roy. Soc.*, B, 1931, **107**, 205). *$\alpha:\alpha'$-Dipyridyl*, 0·5 per cent. in 0·05 *N* sulphuric acid. *Iron standard.* Dissolve 1 g. of pure iron wire in a mixture of 20 ml. of water, 3 ml. of sulphuric acid, and 2 ml. of nitric acid. Evaporate until white fumes appear, and dissolve in 1 litre of water. Prepare other standards by dilution.

Procedure—To 20 ml. of the liquid in a 300-ml. Kjeldahl flask add 1 ml. of sulphuric acid and 5 ml. of nitric acid. Heat until white fumes appear, add 5 ml. of nitric acid, and repeat the heating. At this stage the residue should be a brownish, viscous liquid. Add 1 ml. of perchloric acid (sp.gr. 1·67) and heat until the mixture is colourless. Cool, add 25 ml. of water, boil, cool, and, if necessary, filter through a sintered-glass filter. To this solution add 1 drop of a 1 per cent. solution of *p*-nitrophenol in water and neutralise with the ammonia reagent, followed by 3 ml. in excess. Pass hydrogen sulphide. Filter through a G4 sintered-glass filter (even if no precipitate is perceptible), taking care that the filter is never allowed to become dry. Dissolve the precipitate in 1 ml. of hydrochloric acid, and wash through with 1 ml. of acid followed by 15 ml. of water. Evaporate the filtrate to 10 ml., transfer to a 50-ml. measuring flask, add 1 drop of *p*-nitrophenol solution, and neutralise with sodium hydroxide. Add 2 ml. of buffer solution, followed by 1 ml. of hyposulphite ($Na_2S_2O_4$) solution and 1 ml. of dipyridyl solution. Make up to the mark, allow to stand until the colour has reached its maximum intensity (12 to 24 hours); this may be accelerated by keeping at 37° C. overnight. Determine the absorption at 500 mμ. by means of a photo-electric colorimeter. G. M.

Physical Methods, Apparatus, etc.

Tests for Aluminium Oxide for Chromatographic Analysis. V. A. Christensen (*Dansk Tidsskr. Farm.*, 1945, **19**, 129–148)—The essentials of an alumina suitable for the analysis of alkaloidal salts are that it should retain the acid radical but not the base, and that it should not give up alkali at the alcohol concentration used. The former property may be tested for as follows. Shake 5 g. of the alumina with 20 ml. of a 1 per cent. solution of procaine hydrochloride in 86 per cent. alcohol at frequent intervals during 15 min., filter, and titrate 10 ml. of the filtrate, diluted with an equal volume of water, with 0·1 *N* hydrochloric acid, using bromophenol blue as indicator. To determine the loss of alkali, shake 5 g. at frequent intervals during 15 min. with 20 ml. of water, or of dilute alcohol, or of alcohol, filter, and titrate the filtrate similarly. By these tests a number of samples of alumina, prepared by different methods, were compared. By keeping in a damp place the water content of the alumina may increase from about 5 per cent. to 18·5 per cent., with considerable loss in adsorptive power. Treatment with methyl alcohol also inactivates alumina. G. M.

Chromatographic "Spot" Tests. P. P. Hopf (*J. Chem. Soc.*, 1946, 785–786)—Flood (*Z. anal. Chem.*, 1940, **120**, 327) showed that filter-paper treated with peptised alumina behaved in the same way as a chromatographic column to form a series of rings, each corresponding to one constituent of the mixture, when treated with a suitable developer. It has now been found possible to render visible the rings due to the separated constituents by incorporating the "developer" in the paper. The technique can be used for quantitative analysis.

Procedure—Selection of paper—Dissolve 5 g. of the paper in 5 ml. of sulphuric acid, heat on the hot-plate with sufficient nitric acid to destroy organic matter, dilute to 100 ml., filter, and neutralise with ammonia. There should be no colour change or precipitate on addition of 8-hydroxyquinoline solution. If the paper passes this test, dip a piece into water, 1 inch below the surface and 3 inches above. The water should be drawn up to 2 inches above the surface within 1 min.

Preparation of paper—Dip the paper in a solution of colloidal alumina, prepared by peptising a solution of sodium aluminate with acetic acid (Weiser, *J. Physical Chem.*, 1920, **24**, 525). Alternatively, dip the paper in sodium aluminate solution, dry it, and then dip it in sodium carbonate solution. Wash for 5 hr. at 20° to 25° C. with stirring, soak in the selected developer, wash with water, and air-dry at room temperature.

The following are examples of the application of the method—

Ferromanganese—A drop of a solution containing 3 per cent. of the alloy, adjusted to faint acidity, on a paper prepared with peptised alumina and formaldoxime is allowed to dry on a flat surface and exposed to ammonia vapour.

Ferromolybdenum—A slightly acid, 4 per cent. solution of the alloy, on a paper prepared with peptised alumina and 5 per cent. 8-hydroxyquinoline solution in 10 per cent. acetic acid, gave an outer zone of black (Fe) separated from an inner yellow spot (Mo) by a blank zone.

Alkali metals—An aqueous solution containing 5 per cent. each of sodium acetate, ammonium chloride, and potassium chloride, acidified with 2 per cent. of acetic acid and dropped on to a paper impregnated with a hot, strong starch solution, in place of peptised alumina and with a solution of violuric acid according to Erlenmeyer (*Helv. Chim. Acta*, 1914, **24**, 878) as the developer, gave an inner blank zone followed by a red sodium zone, a pink potassium zone, a blank zone, and a blue ammonia zone.

Aldehydes and ketones—An alumina paper that had been moistened with a 1 per cent. solution of 2:4 dinitrophenyl-hydrazine containing 2 per cent. of acetic acid and dried, gave, with an ethereal solution containing 10 per cent. each of methyl hexyl ketone, benzaldehyde, and acetaldehyde, three zones with benzaldehyde at the centre and acetaldehyde in the outermost position.

From measurements of the radii of the rings obtained in some of these tests the ratio of the amounts of the constituents present can be calculated.

W. S. W.

Reviews

FATTY ACIDS, THEIR CHEMISTRY AND PHYSICAL PROPERTIES. By KLARE S. MARKLEY. Pp. x + 668. New York and London: Interscience Publishers, Inc. 1947. Price 60s.

This is claimed to be the first book devoted solely to an exposition of our knowledge of the fatty acids *per se*; and its avowed object is to bring together in an organised and readily accessible form as much as possible of the facts that have been accumulating for about 150 years on the chemical reactions and physical properties of these compounds, paying attention more especially to the long-chain acids which are the building stones of the natural fats and waxes.

The book is divided into six main sections dealing with the history and nature of fats and waxes, the classification and structure of the fatty acids, their physical properties, chemical reactions and syntheses, and finally their isolation and identification.

A good deal of critical selection has been forced upon the author by reason of the fact that many of the most useful properties were determined a quarter of a century ago, when even moderately pure fatty acids were seldom available; it has evidently proved difficult to decide which value among several recorded by a number of writers is the most reliable, but the author has not shrunk from the task of discarding those he has least faith in. He has provided an extremely useful compendium of information formerly scattered widely, and this very bringing together of the material should, as the author hopes, stimulate others to fill in the gaps in our knowledge that become apparent from reading his book. K. A. WILLIAMS

THE MICROSCOPE, ITS THEORY AND APPLICATIONS. By J. H. WREDDEN, F.R.M.S. Pp. 298 + xxiv. London: J. & A. Churchill, Ltd. 1947. Price 21s.

There would appear to be a definite place in the literature of the microscope and its applications for a book designed to suit the needs of general scientific workers who use a microscope as a means to an end rather than as an end in itself; that is to say, a book that helps, as does this one, to bridge the wide gap that lies between "Carpenter" and the chapters on the microscopy of various substances and materials that appear in books mainly concerned with other methods of examination.

Among those to whom the microscope is often an indispensable working tool is the chemist, and it is to him that this book, written by a chemist, may be expected to appeal more particularly. It includes chapters on elementary optics, the theory of the compound microscope, the optical and illuminating systems, the stand and mechanical parts, the polarising microscope, micrometry, photomicrography and the preparation and mounting of specimens. All are obviously written by one who has had much practice with the many different types of microscope and accessory that are described, as well as with the wide variety of problems that can find their way into an industrial laboratory. Among the most useful sections of the book are those that deal with critical illumination and the methods for obtaining it; on this important, but so often neglected, aspect of setting up a microscope the author sheds a flood of light. The section dealing with the preparation and mounting of specimens, occupying forty pages, is well done, but might with advantage to a book of this kind have been extended to cover a wider range of specimens. In this section, by some curious oversight, there is no mention of how cover glasses may be cleaned. (The medical school method of laying them on the end of a cork and rubbing, first with a moistened duster, with the help of a little acid if necessary, and finally with a dry wash-leather, is worth trying by anyone who finds these fragile objects difficult to handle.)

For the wealth of practical instruction and the information that it contains, this book may be read with profit, but with very little of the pleasure that rewards the reader of a text unblemished by editorial and typographical imperfections. F. L. OKELL

CALCULATIONS OF ANALYTICAL CHEMISTRY. By L. F. HAMILTON and S. G. SIMPSON. Pp. xiii + 387. New York and London: McGraw-Hill Book Co., Inc. 1947. Price 17s. 6d.

Chemical calculations are the delight of some students and the bugbear of many. Since they form an essential part of chemical training both for practical work and for passing examinations, it is desirable that they should be presented in a straightforward and interesting way likely to hold the attention of the many. The present volume does this. The ground covered is divided into seven parts, headed General Analysis, Gravimetric Analysis, Volumetric Analysis, Electrometric Methods, Gas Analysis, Common Analytical Determinations, and Problems of Specific Groups and Determinations. Every phase of general analytical chemistry is covered in the more than 1000 problems, which, as the Preface says, "require reasoning on the part of the student and are not solved by simple substitution in a formula."

The authors are Professor and Associate Professor of Analytical Chemistry in the Massachusetts Institute of Technology, and are to be congratulated on producing a work invaluable for the instruction of students and, dare we say, for refreshing the minds of practising analysts. J. R. NICHOLLS

PHYSICAL METHODS AND BIOLOGICAL METHODS GROUPS

A JOINT meeting of the Physical Methods Group and the Biological Methods Group will be held in the Barnes Hall of the Royal Society of Medicine, 1, Wimpole Street, London, W.1, on Thursday, January 29th, 1948. The subject of the meeting will be "Methods of Penicillin Assay, their Purpose, Scope and Validity." An afternoon session dealing with physical and chemical methods will be held from 2.30 to 4.45 p.m., and an evening session on biological methods will begin at 5.30 p.m.

MICROCHEMISTRY GROUP

THE Third Annual General Meeting of the Microchemistry Group will be held at Imperial College, South Kensington, on Friday, January 30th, 1948.

29 APR 1948

ERRATA:

VOL. 71, 1946:

p. 555. Line 17 from the bottom, *for* "nickel" *read* "nickelous."

VOL. 72, 1947:

p. 253. Line 5 from the bottom, *for* "County of Essex" *read* "County of Sussex."

p. 310. Heading of the third abstract, *for* "J. Warren" *read* "J. W. Sackett."

INDEX TO VOLUME 72

INDEX TO NAMES

* Denotes authors of original papers and notes that have been published in *The Analyst.*

A

Abramson, E., *et al.* Micro-separation of zinc and aluminium by means of potassium ethyl xanthogenate, 76.

Adams, R. S. Ion exchange resins in study of chrome liquors, 169.

***Airey, L.** Application of cathode ray oscillograph to polarography: general lay-out and uses of cathode ray polarograph, 304.

Albanese, A. A., *et al.* Colorimetric estmn. of proteins in body fluids, 208.

Alexander, O. R., *et al.* Polarographic detmn. of tin in foods and biological materials, 111.

***Alexander, W. A.** Colour reaction of certain classes of azo dyes with copper, 56.

Alicino, J. F., Iodimetric assay of penicillin preparations, 68.

***Allerton, F. W.** Detmng. nitrates in water, 349.

Ambler, J. A., *et al.* Quantitative method for aconitic acid and aconitates, 453.

Amos, A. J., *et al.* Modern Cereal Chemistry. 4th Edn. (Review), 462.

Anderson, D. H., *et al.* Rapid electrometric detmn. of alkalinity of sea water, 375.

Anderson, R. C., *et al.* Colorimetric assay of digitoxin by modified Raymond method, 362.

***Andrew, H. G.** Indicator changing at *p*H 0·5 for control of sulphide pptn., 481.

Andrew, R. L., *et al. Alcohol in blood and urine, 21.

— Distribution of barbiturates in human body, 21.

***Angell, F. G.** Analytical methods applicable to furan and its derivatives, 178.

Archibald, R. M. Colorimetric estmn. of canavanine, 25.

Arefyeva, T. V., *et al.* Applying polarographic analysis to production control in non-ferrous metallurgy. I. Detmng. copper, lead and zinc impurities in metallic cadmium. II. Control of solutions and products in cobalt manufacture. III. Detmng. copper, bismuth, lead, cadmium and zinc impurities in metallic tin and lead - tin solders. IV. Detmng. impurities in metallic lead. V. Rapid detmn. of lead, cadmium and zinc in ores and refuse from ore-enrichment (zinc, lead and cadmium ores), 415–417.

Armstrong, A. R., *et al.* Colorimetric detmn. of titanium with disodium-1 : 2-dihydroxy-benzene-3 : 5-disulphonate, 552.

Arnaud, F. W. F. Review of *The Nation's Food: Survey of Scientific Data,* 271.

Asenjo, C. F., *et al.* Puerto Rican fatty oils. VI. Characteristics and composition of "Molinillo" seed oil, 485.

***Ashmore, S. A.** Prepng. *cyclo*hexane for spectroscopic work, 206.

***Austin, G. J.** System of qualitative analysis for common metals in presence of phosphates, using ammonium benzoate, 443.

Avaliani, K. Micro-detmn. of chlorides by means of mercurous iodate, 370.

B

Bacharach, A. L. Review of *Chemical Composition of Foods.* 2nd Edn., 81.

— Review of *Penicillin: its Properties, Uses and Preparations,* 225.

*— Separating crystals and "gums" on micro or semimicro scale, 244.

— ***et al.*** The Nation's Food: Survey of Scientific Data. (Review), 271.

Bagshawe, B., *et al. Detmng. tungsten in steel, using toluene-3 : 4-dithiol. Removal of molybdenum interference by selective extraction, 189.

Bailey, E. D. Particle size by spectral transmission, 222.

Bairstow, S., *et al. Photometric detmn. of dissolved oxygen in condensates and feed waters by means of starch-iodide complex, 340.

Bangerter, F., *et al.* Titration methods for detmng. hardness of water, 322.

Bank, H. N., *et al.* Separating and detmng. primary, secondary and tertiary alkaryl amines (ring-methylated and nitrogen-methylated homologues of aniline), 451.

Banks, C. K., *et al.* Quantitative estmn. of arsenoso group, 265.

***Barnes, H.** Detmng. mercury with dithizone, 469.

*— Detmng. micro-quantities of derivatives of phenarsazine, 241.

Barnett, J., *et al.* Polarographic estmn. of steroid hormones. Polarography of neutral 17-ketosteroids in urinary extracts, 26.

Barraclough, K. C., *et al.* Detmng. hydrogen in liquid steel, 457.

Barrett, E. P., *et al.* Detmng. rates of de-oxidation of iron oxide materials in reducing gases, 318.

Bartels, B., *et al.* Effect of the anaesthetic and rate of injection of digitalis upon lethal dose in cats, 449.

Barthauer, G. L., *et al.* Analytical chemistry of rare earths. Active-oxygen detmn., 217.

Battye, H. G. Obituary, 131.

Baur, F. J., jun., *et al.* Fatty acids of corn (maize) oil, 448.

Bayliss, N. S., *et al.* Thiocyanate complex as means of extracting cobalt before micro-detmn. by other methods, 222.

Beal, G. D., *et al.* Detmng. chlorine in 2 : 2′-dihydroxy-5 : 5′-dichlorodiphenylmethane, 31.

Beaver, J. J., ***et al.*** Tocopherol content of skeletal muscle: comparison of chemical and bio-assay methods, 161.

Bechtel, W. G., ***et al.*** Recording viscometer for starches, 407.

Beck, G. Detcng. ytterbium in rare-earth mixtures, 124.

— New principle of fractionation of rare earths with nitrilo-triacetate. Separating lanthanum and cerium, 74.

Bennett, G. M. Review of *Organic Chemistry*. 3rd English Edn., 421.

Bergdoll, M. S., ***et al.*** Chromatography in separating and detmng. basic amino acids, 77.

Bessey, O. A., ***et al.*** Estmng. vitamin A and carotene in small quantities of blood serum, 161.

Besso, Z., ***et al.*** Micro-separation of zinc and aluminium by means of potassium ethyl xanthogenate, 76.

Beyer, G. L., ***et al.*** Quantitative separation of barium from strontium, 551.

Biedermann, W., ***et al.*** Titration methods for detmng. hardness of water, 322.

Birchard, W. H. Detmng. available chlorine in paper, 493.

Bjornson, O., ***et al.*** Polarographic detmn. of oestrone in urine, 403.

Blackie, J. J. Obituary, 35, 131.

Blohm, C. L., ***et al.*** Simultaneous detmn. of hydrogen sulphide and carbon dioxide in continuous gas stream, 495.

Blum, A. E., ***et al.*** Colorimetric estmn. of methionine in proteins and foods, 210.

— Microbiological estmn. of methionine in proteins and foods, 211.

Bobtelsky, M., ***et al.*** Metallic complexes of tartrates and citrates: structure and behaviour in dilute solutions. I. Cupric and nickelous complexes, 213.

Boizot, G. E. Obituary, 131.

***Bonner, T. G.** Estmng. volatile matter content of propellant explosives. 3. Estmng. acetone, 434.

*— Ditto, ditto. 2. Estmng. ethyl alcohol and ether, 47.

Boorman, E. J.,** ***et al. Identifying phenol from synthetic and natural sources, 246.

***Bowden, S. T.** Micro filter thimble, 542.

***Bowen, E. J.** Fluorescence quenching in solutions, 379.

*— Fluorimeter design, 377.

Bower, R. S., ***et al.*** Chromatography of sugars and their derivatives, 316.

Bradbury, J. T. Detmng. sodium in biological fluids, 112.

Bradford, E. A. M., ***et al.*** Microbiological estmn. of riboflavine in blood, 24.

Brameld, V. E., ***et al.*** Solubility of acetylene in acetone, 31.

Brand, J. C. D. Improved analysis of fuming and concentrated sulphuric acid by water titration, 168.

— Nitration in sulphuric acid. III. Influence of nitric acid and other substances on the oleum-water titration, 169.

Branson, V. C. Appointment as Deputy Public Analyst to County Borough of Hastings, 64; as Deputy Public Analyst for County of Sussex and Borough of Hove, 253.

Brasher, D. M.,** ***et al. Polarographic detmn. of lead in foodstuffs, 423.

Braude, E. A., ***et al.*** Detmng. "active" hydrogen, 213.

Bray, G. T., ***et al.*** Detmng. pyrethrin content of dilute prepns. of pyrethrum flowers in oil, 264.

Breusch, F. L., ***et al.*** Isolating and identifying fatty acids as bis-(p-dimethylaminophenyl)-ureides, 364.

Brightwell, S. T. P., ***et al.*** Detmng. pyrethrins in pyrethrum concentrates in mineral oil, 458.

Brock, M. J., ***et al.*** Estmng. vitamin A and carotene in small quantities of blood serum, 161.

Broughton, D. B., ***et al.*** Explosion in detmng. cobalt as potassium cobaltinitrite, 410.

Brown, A. H. Estmng. pentose in presence of large quantities of glucose, 164.

Brown, E. A. Detmng. iron in presence of cobalt, 122.

Brown, J. B., ***et al.*** Fatty acids of corn (maize) oil, 448.

Browning, B. L., ***et al.*** Disintegration of viscose in analysing paper fibres, 491.

Brownlee, K. A. Industrial Experimentation. 2nd Edn. (Review), 422.

Bruce, A. Obituary, 131, 132.

Brückmann, G. Detmng. alloxan, 24.

Buchanan, J. L. Obituary, 131, 132.

Burchfield, H. P., ***et al.*** Chromatographic resolution of quinone oximes, 77.

Burdett, R. A., ***et al.*** Micro-detmn. of tetra-ethyl lead in gasoline, 454.

Burmaster, C. F. Micro-detmn. of serine and ethanolamine in phospholipid hydrolysates, 23.

Butler, B., ***et al.*** Microbiological estmn. of amino acids. III. Methionine, 209.

Butterfield, W. J. A. Obituary, 131, 132.

C

Caley, E. R., ***et al.*** Behaviour of uranyl solutions in mercury reductor, 216.

***Cambitzi, A.** Formation of racemic calcium tartrate in wines, 542.

Campbell, T. W., ***et al.*** Analysis of organoselenium compounds, 30.

Candlin, E. J. Identifying melamine, 491.

Caraway, K. P., ***et al.*** Ferrous ethylenediamine sulphate as oxidimetric standard, 491.

Carter, R. H. Detmng. DDT in milk by detmng. organic chlorine, 361.

— ***et al.*** Detmng. DDT deposits on fruits, vegetables and vegetation, 401.

***Carter, R. J.** Applications of electrometric methods in analysis, 94.

Cassidy, H. G., ***et al.*** Chromatographic adsorption of amino acids on organic exchange resins, 450.

Chalmers, F. G. D. Appointment as Public Analyst for Urban District of Solihull, 253.

Chapman, G. W. Rapid semi-micro volumetric detmn. of potash in leaf ash, 374.

Chen, K. K., ***et al.*** Colorimetric assay of digitoxin by modified Raymond method, 362.

Chirnside, R. C.,** ***et al. Analysing nickel-cobalt-iron alloys used in glass-to-metal seals, 351.

*— Detmng. lead as iodate in glass analysis, 205.

Chivers, J. E.,** ***et al. New titrimetric estmn. of fluorine, 43.

Christensen, V. A. Tests for aluminium oxide for chromatographic analysis, 556.

Churchman, C. W. Most economical sampling for chemical analysis, 323.

Claassen, A., ***et al.*** Detmng. uranium with 8-hydroxyquinoline, 123.

Claborn, H. V. Detmng. DDT in presence of DDD, 554.

Clardy, F. B., ***et al.*** Detmng. chromium by oxidation with perchloric acid, 410.

Clark, G. L., ***et al.*** X-ray identification and crystallography of aldehydes and ketones as 2 : 4-dinitrophenylhydrazones, 407.

Clark, M. T., ***et al.*** Solubility of acetylene in acetone, 31.

Clark, R. N., ***et al.*** Detmng. silicon in relatively non-volatile organosilicon compounds, 264.

Cleaver, C. S., ***et al.*** Chromatographic adsorption of amino acids on organic exchange resins, 450.

Cluley, H. J.,** ***et al. Analysing nickel - cobalt - iron alloys used in glass-to-metal seals, 351.

Coalstad, S. E. Electrometric estmn. of sucrose and reducing sugars, 409.

Coates, G. E. Improved glass - electrode cell assembly, 418.

Coke, H., ***et al.*** Microbiological estmn. of riboflavine in blood, 24.

Cole, J. W., ***et al.*** Micro-gravimetric detmn. of carbon monoxide, 418.

Cook, A. H. Review of *Organic Preparations*, 33.

Cook, J. H., ***et al.*** Detmng. egg yolk in egg white, 158.

Coombes, A. H. Appointment as Deputy Public Analyst for City of Birmingham, 152.

Cooper, B. S. Review of *Index to the Literature on Spectrochemical Analysis, Part II,* 1940 *to* 1945, 325.

Cowherd, R. D. Application of differential manometer to measurement of fluid density and specific gravity with special reference to temperature effects, 80.

Cox, H. E. Appointment as Public Analyst to Isles of Scilly, 64.

— Review of *Thorpe's Dictionary of Applied Chemistry.* 4th Edn. Vol. VIII. Meth - Oils, Essential, 499.

Craig, A. Factors affecting constancy of analytical weights, 420.

Cram, D. J., ***et al.*** Chemical assay for penicillin G, 313.

Crews, S. K. Review of *Textbook of Pharmacognosy*, 376.

Critchfield, F. H., ***et al.*** Solid polyethylene glycols (Carbowax compounds). Quantitative detmn. in biological materials, 404.

Crossley, H. E., ***et al.*** Analysis of external deposits from boilers. I. General remarks on nature of boiler deposits and their examination, 119.

Cruz, W. O., ***et al.*** Detmng. carbon monoxide in blood, and total and active haemoglobin by carbon monoxide capacity, 257.

Cutter, H. B., ***et al.*** 6-Nitro-2-mercaptobenzothiazole ("Nitro-Captax") as reagent for identifying alkyl halides, 550.

D

Dargie, A. Appointment as Public Analyst to Counties of Angus, Fife, Perth and Kinross, and Burghs of Arbroath, Dundee, Dunfermline and Kirkcaldy, 64.

Daubert, B. F., ***et al.*** Detmng. linoleic acid in cholesteryl linoleate, 314.

Daubney, C. G.,** ***et al. Identifying phenol from synthetic and natural sources, 246.

Davies, O. L., ***et al.*** Accuracy of colorimetric instruments in dye strength detmns., 420.

Davies, W. C. Remarks on polarographs, 273.

—** ***et al. Detmng. copper and arsenic in iron pyrites by internal electrolysis, 17.

Davis, D. W. Isostatic method for detmng. gas permeability of sheet materials, 81.

Dedicoat, H. Appointment as Deputy Public Analyst and Deputy Official Agricultural Analyst for County Borough of Leeds, 253, 254.

— Appointment as Deputy Public Analyst to County Borough of Leeds not taken up, 397.

de Goeij, H. J. A. Phase-volumetric analysis of mixtures of benzine and aromatic hydrocarbons, 549.

Deijs, W. B., ***et al.*** Detmng. traces of copper in vegetable material with diethyldithiocarbamate, 555.

Deinum, H. W. Purifying carbon rods for spectrochemical analysis. III, 419.

de Lange, J. J., ***et al.*** Identifying crystalline organic compounds by X-ray analysis, 406.

Delaune, R. H., ***et al.*** Separating calcium from magnesium by oxalate method in samples of high magnesium - calcium ratio, 118.

de Leenheer, L., ***et al.*** Detmng. quartz content of clay fraction of soils, 217.

Dermanova, I. G., ***et al.*** Photometric detmn. of bismuth in copper, 265.

Dick, R., ***et al.*** Entrainment of cobalt and sulphur in iron separations, 122.

Diehl, H., ***et al.*** Detmng. silicon in relatively non-volatile organosilicon compounds, 264.

Dische, Z. New specific colour reaction of hexuronic acids, 365.

Dixon, F. Appointment as Deputy Public Analyst for City of Manchester, 397.

Dodd, F. R. Obituary of W. H. Roberts, 36.

Dodge, B. F., ***et al.*** Colorimetric detmn. of traces of carbon dioxide in air, 418.

Dorée, C. Methods of Cellulose Chemistry. 2nd Edn. (Review), 422.

Dotts, W. M. Measuring distribution of particle size in dispersed systems, 324.

Doty, D. M., ***et al.*** Chromatography in separating and detmng. basic amino acids, 77.

Dracass, W. R. Obituary, 131, 132.

Dratschev, S. M. Colorimetric detmn. of hardness of water, 460.

Dubovitzkaja, E. I., ***et al.*** Applying polarographic analysis to production control in non-ferrous metallurgy. I. Detmng. copper, lead and zinc impurities in metallic cadmium. II. Control of solutions and products in cobalt manufacture. III. Detmng. copper, bismuth, lead, cadmium and zinc impurities in metallic tin and lead - tin solders. IV. Detmng. impurities in metallic lead. V. Rapid detmn. of lead, cadmium and zinc in ores and refuse from ore-enrichment (zinc, lead and cadmium ores), 415–417.

Duffield, W. D.,** ***et al. Estmng. mercury compounds in the atmosphere, 11.

Dufrenoy, J., ***et al.*** Three-hour physical development cup-plate assay for penicillin, 488.

Duggan, R. E. Estmng. chicken manure in egg products from uric acid content, 400.

— ***et al.*** Indole in shrimp, 545.

Dyson, G. M. Dysonian notation, 155.

— New Notation and Enumeration System for Organic Compounds. (Review), 176.

E

Eble, T. E., ***et al.*** Chemical assay of penicillin, 488.

Edwards, A. H. Analysis of external deposits from boilers. II. Analysing deposits rich in phosphates, 119.

— ***et al.*** Analysis of external deposits from boilers. I. General remarks on nature of boiler deposits and their examination. III. Analysing water-soluble fraction of the deposits, 119.

Edwards, G. W. Obituary, 131, 132.
Eglof, W. K., *et al.* Detmng. calcium in magnesite and fused magnesia, 168.
Ellis, G. H., *et al.* Estmng. cobalt in biological materials with nitrocresol, 113.
Ellison, R. Obituary, 229.
Elvehjem, C. A., *et al.* Hydrolysis procedures for estmng. tryptophan in proteins and foodstuffs by microbiological procedure, 25.
— Microbiological estmn. of methionine in proteins and foodstuffs, 69.
Elving, P. J., *et al.* Analysing mixtures of mercurous and mercuric mercury and sulphuric acid, 493.
Emmerie, A. Colorimetric estmn. of tocopherol, 209.
***Ethrington, C. G.**, *et al.* Detmng. small quantities of manganese in caustic soda, 472.
***Evans, B. S.** Titration of minute amounts of nickel, 110.
*— *et al.* Spot-tests for detcng. alloying elements in lead-base alloys, 105.
*— Spot-tests for detcng. alloying elements in tin-base alloys, 439.
*— Spot-tests for detcng. alloying elements in zinc-base alloys, 101.
***Evans, D. P.** Polarisation end-points, 99.

F

Fainberg, S. J., *et al.* Polarographic detmn. of lead in lead slags, 268.
Falkova, O. B., *et al.* Spectro-analytical detmn. of boron in steel, 411.
Farmer, E. H., *et al.* Simple laboratory falling-film molecular still, 174.
***Fearon, W. R.** Zinc urate oxidation reaction, selective test for zinc, 541.
Felsing, W. A., *et al.* General Chemistry. 2nd Edn. (Review), 226.
Ferber, K. H. Indicator properties of derivatives of 4′-nitrophenylazo-1-naphthol, 72.
Field, E., *et al.* Conversion of organic sulphur to hydrogen sulphide for analysis, 115.
— Detmng. hydrogen sulphide in gases, 172.
— Identifying sulphur compounds in gas mixtures, 219.
Fielding, J. Estmng. penicillin and other bacteriostatics, 256.
***Fieller, E. C.** Statistical background in bio-assay, 37.
***Fill, M. A.**, *et al.* Simple direct-reading semi-micro balance, 394.
Fireman, P. Oxidation of certain metallic hydroxides by a current of air, 214.
Firth, H. Obituary, 131, 132.
Fischbach, H., *et al.* Chemical assay of penicillin, 488.
Fisher, A., *et al.* Colorimetric detmn. of pyridoxine, pyridoxal and pyridoxamine, 260.
Fisher, H. J., *et al.* Detmng. sulphathiazole and other sulpha drugs in honey, 448.
Flagg, J. F., *et al.* Detmng copper in cast iron and steels with quinaldic acid, 121.
Fleming, A., *et al.* Estmng. penicillin in serum. Use of glucose, phenol red, and serum water, 548.
Flint, D., *et al.* Analysis of external deposits from boilers. III. Analysing water-soluble fraction of the deposits, 119.
Flint, J. W. Appointment as Deputy Official Agricultural Analyst for Administrative County of Kent, 153.
Fogelson, E. I., *et al.* Detmng. aluminium in high alloy steel, 493.
— Phenylanthranilic acid in volumetric detmn. of molybdenum in steel and ferro-molybdenum, 413.
Forrest, J., *et al.* Chemical investigations of the insecticide DDT and analogues. I. Reactions of DDT and associated compounds, 218.
***Foster, G. E.** Assay of solutions of *d*-tubocurarine chloride for injection, 62.
Foster, J. A. Obituary, 131, 132.
Fox, S. H., *et al.* Chemical estmn. of nicotinic acid, 362.
***Francis, J.**, *et al.* Photometric detmn. of dissolved oxygen in condensates and feed waters by means of starch-iodide complex, 340.
Frankland, P. F. Obituary, 131, 132.
Freund, H., *et al.* Fractional separation of hafnium and zirconium by means of triethyl phosphate, 216.
Friedman, H., *et al.* Detmng. tetra-ethyl lead in gasoline by X-ray absorption, 269.
Front, J. S., *et al.* Detmng. linoleic acid in cholesteryl linoleate, 314.
Fullam, E. F., *et al.* Prepng. powdered materials for electron microscopy, 224.

G

Gabrio, B. W., *et al.* Precision method for quantitative detmn. of calcium in blood plasma, 310.
Gakenheimer, W. C., *et al.* Assay for choline chloride in pharmaceutical products, 23, 486.
Gambrill, C. M., *et al.* Picric acid detmn. of aromatic content of aviation gasoline, 73.
Garbutt, C. D. Obituary, 131.
Gatos, H. C., *et al.* Detmng. gold with ascorbic acid, 123.
Gegetschkori, N. M., *et al.* Spectro-analytical detmn. of boron in steel, 411.
Gengrinovitsch, A. I., *et al.* Cacotheline in volumetric analysis. I. Detmng. calcium salts by titration with sodium oxalate. II. Detmng. tervalent iron by titration with stannous chloride solution, 492.
Gentry, C. H. R., *et al.* Absorptiometric detmn. of silicon in steels, 121.
Georges, L. W., *et al.* Chromatography of sugars and their derivatives, 316.
Gerritsma, K. W., *et al.* Combined micro-gravimetric and titrimetric micro-detmn. of thallium in toxicological material, 221.
***Gilbert, F. C.** Detmng. calcium oxide in low-lime refractory magnesia, 482.
Giles, C. H., *et al.* Accuracy of colorimetric instruments in dye strength detmns., 420.
Gillham, E. W. F. Automatic detmn. of sulphur trioxide in flue gas, 322.
— Detmng. oxides of sulphur in flue gas, 321.
Gilman, H., *et al.* Detmng. silicon in relatively non-volatile organosilicon compounds, 264.
Ginsburg, N., *et al.* Quantitative analysis of isomeric cresols and cresol-phenol mixtures by U.V. absorption spectra of vapours, 264.
***Glynn, E.** Reductometric detmn. of sulphoxide and amine oxide groups, 248.
Godar, E. M., *et al.* Polarographic detmn. of tin in foods and biological materials, 111.
Golden, H. R., *et al.* 6-Nitro-2-mercaptobenzothiazole ("Nitro-Captax") as reagent for identifying alkyl halides, 550.
Goldstone, N. I. Source of error in Gutzeit method for arsenic, 159.

Goodall, R. R., *et al. Micro-chromatographic detcn. and approximate detmn. of the different penicillins in a mixture, 277.

Gooden, E. L., *et al.* Prepng. standard powders for reference in particle-size measurement, 270.

***Gooderham, W. J.** Apparatus for gas analysis by soap film method, 520.

Goodman, W. Colorimetric detmn. of iron in brass and bronze, 494.

Goodwin, T. W., *et al.* Spectrophotometric detmn. of tyrosine and tryptophan in proteins, 260.

Gordon, B. E., *et al.* Micro-detmn. of tetra-ethyl lead in gasoline, 454.

Gorjuschina, V. G., *et al.* Bivalent chromium solution for volumetric detmn. of tungsten. Detmng. tungsten in tungsten concentrates and ferro-tungsten, 371.

Gottlieb, K. R., *et al.* Chromatographic analysis of alkaloidal salts, 548.

— Limit tests for impurities, 485.

— Limit Tests for Impurities. (Review), 500.

Goyan, F. M., *et al.* Three-hour physical development cup-plate assay for penicillin, 488.

Goyco, J. A., *et al.* Puerto Rican fatty oils. VI. Characteristics and composition of "Molinillo" seed oil, 485.

Graff, J. H., *et al.* Disintegration of viscose in analysing paper fibres, 491.

Graham, R. P. Detmng. free acid in presence of aluminium salts, 122.

Grant, J. Review of *Forensic Chemistry*, 127.

— Review of *Methods of Cellulose Chemistry*. 2nd Edn., 421.

Grant, W. M. Colorimetric micro-detmn. of formic acid, 490.

— ***et al.*** Iodimetric micro-titration for mustard gas, 315.

Greene, L. W. Chemical microscopy of essential oils. IV. Cassia and cinnamon oils, 489.

Greenhut, I. T., *et al.* Hydrolysis procedures for estmng. tryptophan in proteins and foodstuffs by microbiological procedure, 25.

Griffith, L., *et al.* Internal standard method of spectrographic analysis as applied to detmng. lead in high-purity zinc, 223.

Grindley, D. N. Seed oils of some Sudan Caesalpinioideae, 111.

Griswald, J., *et al.* Analysing furfural-water solutions, 165.

Grossowicz, N., *et al.* Microbiological estmn. of nicotinic acid based on use of *Proteus* HX19, 362.

Gulbransen, E. A., *et al.* Electron diffraction and electron microscopic study of oxide films formed on metals and alloys at moderate temperatures, 269.

— Oxide films formed on alloys at moderate temperatures. Electron diffraction and electron microscope study, 223.

Gullstrom, D. K., *et al.* Chromatographic resolution of quinone oximes, 77.

Gunstone, F. D., *et al.* Low temperature crystallisation in detmng. component acids of liquid fats. II. Fats which contain linolenic as well as linoleic and oleic acids, 402.

H

Hakim, D. N., *et al. Chromatographic adsorption method for estmng. provitamin-A content of foodstuffs, 194.

Hale, F., *et al.* Hydrogen-peroxide-treated peptone in media for microbiological detmn. of amino acids, 70.

Hale, F., *et al.* Microbiological estmn. of amino acids. III. Methionine, 209.

Hall, R. T., *et al.* Detmng. conjugated diolefines with chloromaleic anhydride, 164.

Haller, H. L., *et al.* Colorimetric detmn. of DDT in milk and fatty materials, 360.

Haller, R. Detcng. alkaline earth metals in cell tissue of plants, 71.

***Halpern, G. R.** Spectrophotometric changes during oxidation of vitamin A oils, 78.

Hamilton, L. F., *et al.* Calculations of Analytical Chemistry. (Review), 558.

Harbottle, G., *et al.* Detmng. aromatic amines and substituted ureas in smokeless powder. Volumetric bromination procedure, 73.

Hardy, R. A., *et al.* Chromatographic adsorption of amino acids on organic exchange resins, 450.

Hartley, F. Review of *Pharmacopoeia of the United States of America.* 13th Revision (U.S.P. XIII), 498.

Hawkins, E. S. Appointment as Public Analyst and as Official Agricultural Analyst to County Borough of Canterbury, 152, 153; and as Public Analyst to Boroughs of Folkestone, Gravesend, Maidstone and Margate, 64.

Hawley, J. W. Appointment as Public Analyst to Counties of Dumfries, Kirkcudbright and Wigtown, and Burgh of Dumfries, 64.

Hazel, W. M., *et al.* Detmng. calcium in magnesite and fused magnesia, 168.

Heard, R. D. H., *et al.* Colorimetric estmn. of reducing steroids, 71.

Hein, F., *et al.* Volumetric detmn. of organic lead compounds, 549.

Heines, V. Micro-detmn. of pentoses by bisulphite method, 267.

Henly, A. A., *et al.* Polarographic estmn. of steroid hormones. Polarography of neutral 17-ketosteroids in urinary extracts, 26.

Henriques, F. C., jun., *et al.* Radioactive studies. Analytical method for detmng. long-life carbon ^{14}C, 80.

— Ditto, ditto. Analytical procedure for measurement of long-lived radioactive sulphur ^{35}S, with Lauritzen electroscope, and comparison of electroscope with special Geiger counter, 80.

— Ditto, ditto. Analytical procedure for measurement of radioactive arsenic of 90-day half-life, 80.

— Ditto, ditto. Utilisation of radioactive isotope dilution procedures for special types of chemical problems. Detmng. the three individual components of mixtures of dibenzyl-sulphide, -sulphoxide and -sulphone, 80.

Henry, A. J., *et al. Chromium trioxide test for cocaine hydrochloride, 253.

Herd, M. Appointment as Additional Public Analyst to Burgh of Glasgow, 64.

***Heron, A. E.** Oxidation of nitrogen during micro-combustion of organic substances, 142.

Heron, N. Appointment as Deputy Official Agricultural Analyst to County Boroughs of Birkenhead, Blackburn, Bootle and Southport, 543; and as Deputy Public Analyst for Borough of Gosport, and Deputy Official Agricultural Analyst for County Borough of Liverpool, 397.

Herrington, K. D., *et al.* Precision capillarimeter, 460.

Herzfeld, E. Selective cerium reactions, 414.

Heuser, G. F., *et al.* Chemical estmn. of riboflavine, 24.

***Heyrovský, J.** Fundamental laws of polarography, 229.

Heywood, A. D. Obituary, 131, 132.

Hickman, J. W., *et al.* Electron diffraction and electron microscopic study of oxide films formed on metals and alloys at moderate temperatures, 269.
— Oxide films formed on alloys at moderate temperatures. Electron diffraction and electron microscope study, 223.
Higgs, D. G., *et al. Spot-tests for detcng. alloying elements in lead-base alloys, 105.
*— Spot-tests for detcng. alloying elements in tin-base alloys, 439.
*— Spot-tests for detcng. alloying elements in zinc-base alloys, 101.
***High, J. H.** Detmng. copper in food, 60.
Hilditch, T. P. Chemical Constitution of Natural Fats. 2nd Edn. (Review), 500.
— ***et al.*** Low temperature crystallisation in detmng. component acids of liquid fats. I. Fats in which oleic and linoleic acids are major components. II. Fats which contain linolenic as well as linoleic and oleic acids. III. Fats which contain elæostearic as well as linoleic and oleic acids, 402.
*— Spectrophotometric detmn. of small proportions of linolenic acid in fats, 527.
Hill, F. W., *et al.* Chemical estmn. of riboflavine, 24.
Hiller, A., *et al.* Application of Sendroy's iodimetric chloride titration to protein-containing fluids, 364.
— Detmng. carbon monoxide in blood, and total and active haemoglobin by carbon monoxide capacity, 257.
Hinks, E. Obituary, 131, 132.
Hoeke, F. Detmng. the sugar substitute 1-*n*-propoxy-2-amino-4-nitrobenzene, 548.
Holck, H. G. O., *et al.* Effect of the anaesthetic and rate of injection of digitalis upon its lethal dose in cats, 449.
Hollander, M., *et al.* Detmng. boric oxide in glass, 317.
Hoogland, P. L. Purifying carbon rods for spectrochemical analysis. I. Simple apparatus for purification by means of resistance heating, 419.
***Hooper, J. H. D.** Analysing hydrocarbon gases by low temperature distillation, 513.
Hopf, P. P. Chromatographic "spot" tests, 556.
Hopkins, Sir Frederick G. Obituary, 229.
Horn, M. J., *et al.* Colorimetric estmn. of methionine in proteins and foods, 210.
— Microbiological estmn. of methionine in proteins and foods, 211.
Horwitz, W. Modified Scharer phosphatase test for milk and soft cheeses, 544.
Hoskins, J. L., *et al. Estmng. traces of mercury in urine, 6.
Houtman, J. P. W., *et al.* Identifying crystalline organic compounds by X-ray analysis, 406.
***Howard, B. H.** Detmng. small amounts of hexachloro*cyclo*hexane (benzene hexachloride), 427.
***Howes, H. S.** Evaporation from beakers with radiant heater, 111.
*— Simple method for electric heating, 110.
Hoyt, L. F. Qualitative method for detcng. surface-active agents, 270.
Hubanks, P. E., *et al.* Detmng. DDT deposits on fruits, vegetables and vegetation, 401.
Huff, J. W., *et al.* Detmng. N′-methylnicotinamide in urine, 363.
— Rapid fluorimetric estmn. of total pyridine nucleotides in red blood cells, 363.
Hughes, J. W., *et al. Detmng. small quantities of manganese in caustic soda, 472.
Hutchinson, W., *et al. Detmng. nitromethane in air, 432.
*— Polarographic detmn. of tin, lead and zinc in phenol, 149.

I

Ingold, W. Micro-titration of organic acids, 114.
Irby, V., *et al.* Colorimetric estmn. of proteins in body fluids, 208.
Isherwood, F. A. Detmng. and isolating organic acids in fruit, 261.
Ivarsson, B., *et al.* Analysing dilute melamine resin solutions, 32.

J

James, E. M., *et al. Chemical estmn. of nicotinic acid in cereals and other foods, 327.
Jamieson, A. R. Appointment as Public Analyst to Burgh of Glasgow, 64.
Janse, L. C. Fat detmn. in dairy products, 485.
Jansen, N. F., *et al.* Detmng. specific gravity of extracts of jams, syrups, etc., based on density gradient, 399.
Janssen, C. Detmng. dissolved oxygen in presence of sulphite, 496.
Jasper, J. J., *et al.* Precision capillarimeter, 460.
Jenkins, D. C. E. Appointment as Public Analyst to Borough of Colne, 64.
Jenkins, D. M., *et al.* Detmng. chlorine in 2 : 2′-dihydroxy-5 : 5′-dichlorodiphenylmethane, 31.
Jennings, W. P., *et al.* Amperometric titration of cyanide with silver nitrate, using rotating platinum electrode, 319.
Jensen, F. W., *et al.* High-frequency oscillators in titrations and analyses, 78.
Jessop, G. Notes on polarographic technique, 273.
Johnson, C. M., *et al.* Detmng. free fatty acids in dried egg powders, 67.
Jones, D. B., *et al.* Colorimetric estmn. of methionine in proteins and foods, 210.
— Microbiological estmn. of methionine in proteins and foods, 211.
Jones, F. R., *et al. Polarographic detmn. of lead in foodstuffs, 423.
Jones, P. H. Obituary, 131, 132.
Jordan, J., *et al.* Metallic complexes of tartrates and citrates; structure and behaviour in dilute solutions. I. Cupric and nickelous complexes, 213.
Judy, J. N., *et al.* Chromatographic resolution of quinone oximes, 77.
Juntunen, J., *et al.* Three-hour physical development cup-plate assay for penicillin, 488.

K

Kahn, A., *et al.* Micro-detmn. of gaseous olefines, 418.
Kallmann, S. Detmng. sodium and potassium in silicates, 167.
Kalmikova, N. V., *et al.* Detmng. aluminium in high alloy steel, 493.
— Phenylanthranilic acid in volumetric detmn. of molybdenum in steel and ferro-molybdenum, 413.
Kaloyereas, S. A. Rancidity of olive oils, 256.
Kammerer, O. F., *et al.* Photometric detmn. of total cholesterol in plasma or serum by modified Liebermann-Burchard reaction, 27.
Karjakin, J. V., *et al.* Rapid detmn. of sulphate impurities in cryolite, 266.

Karnovsky, M. L., ***et al.*** South African fish products. XXII. Periodic acid as reagent in analysis of unsaponifiable fraction of natural fats, 22.

Karrer, P. Organic Chemistry. 3rd English Edn. (Review), 421.

Kaunitz, H., ***et al.*** Tocopherol content of skeletal muscle: comparison of chemical and bio-assay methods, 161.

Kaye, W. I., ***et al.*** X-ray identification and crystallography of aldehydes and ketones as 2 : 4-dinitrophenylhydrazones, 407.

Keenan, G. L. Microscopical differentiation of common mustard seeds with polarised light, 448.

— Microscopical identification of butacaine sulphate (Butyn) and procaine hydrochloride (Novocaine), 547.

Keilin, B., ***et al.*** Polarographic analysis of nitrite and nitrite - nitrate mixtures, 268.

Kenney, E. F., Raw sugar: sampling, mixing and testing, 402.

Kent-Jones, D. W., ***et al.*** Modern Cereal Chemistry. 4th Edn. (Review), 462.

Kesler, C. C., ***et al.*** Recording viscometer for starches, 407.

Key, C.,** ***et al. Detmng. copper and arsenic in iron pyrites by internal electrolysis, 17.

Kimura, K. K., ***et al.*** Effect of the anaesthetic and rate of injection of digitalis upon its lethal dose in cats, 449.

Kinsey, V. E., ***et al.*** Iodimetric micro-titration for mustard gas, 315.

Kirkbride, J. N., ***et al.*** Internal standard method of spectrographic analysis as applied to detmng. lead in high-purity zinc, 223.

Kirkpatrick, H. F. W. Polarographic study of alkaloids. V, 409.

Kirtchik, H. Detmng. nickel in cobalt steel and alloys, 553.

Kistiakowsky, G. B., ***et al.*** Radioactive studies. Analytical procedure for measurement of long-lived radioactive sulphur ^{35}S, with Lauritzen electroscope, and comparison of electroscope with special Geiger counter, 80.

Klecka, M. E., ***et al.*** Analysing furfural - water solutions, 165.

Klein, A., ***et al.*** Volumetric detmn. of organic lead compounds, 549.

Kline, L., ***et al.*** Detmng. free fatty acids in dried egg powders, 67.

Kniphorst, L. C. E. Colorimetric detmn. of iron and manganese in foods, 446.

— Semi-micro detmn. of calcium in foodstuffs, 159.

— ***et al.*** Detmng. traces of copper in vegetable material with diethyldithiocarbamate, 555.

***Kodicek, E.** Applications of fluorimetry in vitamin analysis, 385.

Kokorin, A. I. Colorimetric detmn. of antimony, 319.

— ***et al.*** Photometric detmn. of bismuth in copper, 265.

Kononiuk, R. T. Pyrophosphoric acid in detmng. nickel in cast iron and steel, 413.

Korinfski, A. A. Detmng. cyanamide derivatives in complex mixtures, 368.

— Qualitative analysis of mixtures of cyanamide derivatives, 367.

Krajčinović, Matya. Identifying acidic oxycellulose by means of aromatic amines and diamines, 366.

Krajčinović, Mladen. Ditto, ditto, 366.

Kramer, P. J. G., ***et al.*** Detmng. narceine in narceine sodium salicylate with Reinecke's salt, 547.

Krilanovich, N. J., ***et al.*** Analysis of organo-selenium compounds, 30.

Kutschment, M. L., ***et al.*** Cacotheline in volumetric analysis. I. Detmng. calcium salts by titration with sodium oxalate. II. Detmng. tervalent iron by titration with stannous chloride solution, 492.

L

LaClair, J. B. Detmng. 1 : 2-*bis*(p′-chlorophenyl)-ethane in DDT dusts and oil solutions, 170.

Laing, M. E., ***et al.*** Explosion in detmng. cobalt as potassium cobaltinitrite, 410.

Laitinen, H. A., ***et al.*** Amperometric titration of cyanide with silver nitrate, using rotating platinum electrode, 319.

— Detmng. hydroperoxides in rubber and synthetic polymers, 369.

Lang, R. Volumetric detmn. of mixtures of ferro-cyanide and arsenious acid, 551.

Lapin, N. N. Silver sulphide method for detmng. cadmium, 373.

***Lauchlan, A. D. E.** Recent developments in apparatus for *p*H measurements and electro-titrations, 90.

Lauer, J. L., ***et al.*** Quantitative analysis of hydro-carbon mixtures by means of Raman spectra, 261.

***Lea, C. H.** Chapman and McFarlane method for estmng. reducing groups in milk powder, 336.

— Detmng. peroxide values of edible fats and oils. Iodimetric method, 255.

Lee, J. H. Detmng. extinction corrections in infra-red analysis of gaseous hydrocarbon mixtures, 224.

Lees, H. Simple automatic percolator, 496.

LeRoy, D. J., ***et al.*** Micro-detmn. of gaseous olefines, 418.

Levi, A. A.,** ***et al. Micro-chromatographic detcn. and approximate detmn. of the different penicillins in a mixture, 277.

Levitas, N., ***et al.*** Rapid fluorimetric estmn. of total pyridine nucleotides in red blood cells, 363.

Levy, G. B., ***et al.*** Polarographic analysis of streptomycin, 310.

***Lewis, J. A.** Aids to accuracy in quantitative estmn. from simple polarograms, 308.

Liddell, H. F.,** ***et al. New titrimetric estmn. of fluorine, 43.

Liebhafsky, H. A., ***et al.*** Traces of oxygen in hydrogen or nitrogen, 267.

Lindstrom, E. G., ***et al.*** Colour reactions of un-symmetrical diarylethylenes, 489.

Lingane, J. J. Operating characteristics of Sargent model XX visible recording polarograph, 496.

— Polarographic investigations of oxalate, citrate and tartrate complexes of ferric and ferrous iron, 370.

— ***et al.*** Extracting vanadium from aqueous solutions by *iso*propyl ether, 216.

Liverseege, J. F. Obituary, 131, 132.

Lochvitzkaja, A. P., ***et al.*** Colorimetric detmn. of molybdenum as thiocyanate complex, 455.

Lopez, J. A., ***et al.*** Estmng. vitamin A and carotene in small quantities of blood serum, 161.

Lord, K. A., ***et al.*** Detmng. pyrethrin content of dilute prepns. of pyrethrum flowers in oil, 264.

Lovecy, A. Improved "Kjeldahl trap" and ammonia distillation apparatus, 420.

Lovett, T. W. Appointment as Deputy Public Analyst to County Borough of Rochdale, 64; and County Borough of Oldham, 253.

Lowry, O. H., ***et al.*** Estmng. vitamin A and carotene in small quantities of blood serum, 161.

Lyman, C. M., *et al.* Hydrogen-peroxide-treated peptone in media for microbiological detmn. of amino acids, 70.
— Microbiological estmn. of amino acids. III. Methionine, 209.

M

McAlpine, R. K. Anomalous behaviour of some oxidising agents, 75.
— Sensitivity of pptn. reactions in dilute electrolytes, 116.
McCance, R. A., *et al.* Chemical Composition of Foods. 2nd Edn. (Review), 81.
McCullough, J. D., *et al.* Analysis of organoselenium compounds, 30.
Macdonald, J. Appointment as Assistant Public Analyst to Burgh of Aberdeen, 64.
MacEwan, D. M. C. Identifying and estmng. montmorillonite group of minerals, with special reference to soil clays, 218.
McGrath, T. Obituary, 131.
McKean, J. B. Appointment as Public Analyst to Counties of Argyll, Bute, Dumbarton, Stirling and Lanark, and Burghs of Greenock, Port Glasgow, Paisley, Dumbarton, Motherwell, Wishaw, Airdrie, Clydebank, Coatbridge, Falkirk, Hamilton, Perth, Rutherglen and Stirling, 64.
McVeigh, J. L., *et al.* 2 : 4-Dinitrophenylsemicarbazide. Reagent for aldehydes and ketones, 212.
Mader, W. J., *et al.* Chemical assay for penicillin G, 313.
Mandel, J. Improvement of precision by repeated measurements. Application to analytical control methods, 324.
Mandl, I., *et al.* Identifying fructose, 263.
***Mansour, R.,** *et al.* Chromium trioxide test for cocaine hydrochloride, 253.
Marcali, K., *et al.* Kjeldahl detmn. of nitrogen. Eliminating distillation, 163.
Margnetti, C., *et al.* Radioactive studies. Analytical method for detmng. long-life carbon ^{14}C, 80.
— Ditto, ditto. Analytical procedure for measurement of long-lived radioactive sulphur ^{35}S, with Lauritzen electroscope, and comparison of electroscope with special Geiger counter, 80.
— Ditto, ditto. Analytical procedure for measurement of radioactive arsenic of 90-day half-life, 80.
— Ditto, ditto. Utilisation of radioactive isotope dilution procedures for special types of chemical problems. Detmng. the three individual components of mixtures of dibenzyl-sulphide, -sulphoxide and -sulphone, 80.
Markley, K. S. Fatty Acids, their Chemistry and Physical Properties. (Review), 557.
Marsh, J. K. Rare-earth metal oxides. II. Study of oxide pptn. by nitrate fusion, 169.
— Ditto, ditto. III. Pptn. from potassium hydroxide melts, 169.
***Martin, A. E.,** *et al.* Estmng. 2 : 2-*bis*-(*p*-chlorophenyl)-1 : 1 : 1-trichloroethane (*p,p′*-DDT) by dehydrohalogenation, 1.
*— Identifying phenol from synthetic and natural sources, 246.
Martin, C. C., *et al.* Quantitative analysis of hydrocarbon mixtures by means of Raman spectra, 261.
Martin, J. B., *et al.* Picric acid detmn. of aromatic content of aviation gasoline, 73.
Martin, J. T., *et al.* Detmng. pyrethrins in pyrethrum concentrates in mineral oil, 458.
Martinez-Pico, Z., *et al.* Puerto Rican fatty oils. VI. Characteristics and composition of "Molinillo" seed oil, 485.
Mason, F. A. Obituary, 423.
Matsen, F. A., *et al.* Quantitative analysis of isomeric cresols and cresol - phenol mixtures by U.V. absorption spectra of vapours, 264.
Matthews, F. W., *et al.* Identifying organic compounds by use of chromium target X-ray diffraction powder patterns, 174.
Maxwell, W. H. Current Waterworks Practice: Practical Treatise on Provision of Water Supplies for Urban and Rural Communities. (Review), 270.
***Mayer, A.,** *et al.* Direct colorimetric detmn. of tungsten in cast iron, 464.
Mee, A. J. Translator of *Organic Chemistry*, Karrer, 3rd English Edn. (Review), 421.
Meesook, B., *et al.* Comparative estmns. of carbonyl and carboxyl groups in chromium trioxide and hypochlorous acid oxycelluloses and oxyxylans, 72.
Meggers, W. F., *et al.* Index to the Literature on Spectrochemical Analysis, Part II, 1940 to 1945. (Review), 325.
Mehl, J. W., *et al.* Detmng. serum phosphate by molybdi-vanadate method, 312.
Mehlenbacher, V. C., *et al.* Detmng. egg yolk in egg white, 158.
Meites, L., jun., *et al.* Extracting vanadium from aqueous solutions by *iso*propyl ether, 216.
Melling, S. E. Review of *Current Waterworks Practice : Practical Treatise on Provision of Water Supplies for Urban and Rural Communities,* 270.
Mesée, H. J., *et al.* Volumetric detmn. of organic lead compounds, 549.
Michell, J. H., *et al.* Identifying organic compounds by use of chromium target X-ray diffraction powder patterns, 174.
Middleton, G. Review of *Limit Tests for Impurities,* 500.
Miller, R. R., *et al.* Analysis of boron trifluoride in organic liquids (ethers), 32.
— Routine analysis of sodium - potassium alloys, 317.
***Milton, R. F.,** *et al.* Estmng. mercury compounds in the atmosphere, 11.
*— Estmng. traces of mercury in urine, 6.
*— New titrimetric estmn. of fluorine, 43.
Moir, D. D. Appointment as Public Analyst to Urban District of Coulsdon and Purley, 64.
***Monier-Williams, G. W.** Address of Retiring President, 137.
Monnier, D., *et al.* Colorimetric detmn. of fluoride, 117.
— Semi-quantitative inorganic analysis, 116.
Morell, D. B., *et al.* Fluorimetric estmn. of riboflavine in urine, 257.
— Modification of fluorimetric method of estmng. riboflavine in biological materials, 257.
Morrell, R. S. Obituary, 35, 131, 133.
***Morris, C. J. O. R.** Rotating platinum electrode, 298.
— *et al.* Polarographic estmn. of steroid hormones. Polarography of neutral 17-ketosteroids in urinary extracts, 26.
Morris, D. L. Colorimetric estmn. of glycogen, 164.
Morton, R. A., *et al.* Spectrophotometric detmn. of tyrosine and tryptophan in proteins, 260.
Moseley, O., *et al.* Hydrogen-peroxide-treated peptone in media for microbiological detmn. of amino acids, 70.
— Microbiological estmn. of amino acids. III. Methionine, 209.

Moss, M. L., *et al.* Detmng. conjugated diolefines with chloromaleic anhydride, 164.

Mueller, A., *et al.* Chemical estmn. of nicotinic acid, 362.

Mulder, E. G., *et al.* Detmng. traces of copper in vegetable material with diethyldithiocarbamate, 555.

Mundell, M., *et al.* Chemical assay of penicillin, 488.

Mundy, L. M. Appointment as Public Analyst to Counties of Ayr and Renfrew, and Burghs of Ayr and Kilmarnock, 64.

Muraschova, V. I., *et al.* Rapid detmn. of sulphate impurities in cryolite, 266.

N

Naidu, S. R. Obituary, 177.

Needs, F. E. Obituary, 131, 133.

Nelson, J. S., *et al.* Detmng. hydroperoxides in rubber and synthetic polymers, 369.

***Nelson, W. A. G.** Detmng. carotene in dried grass, 200.

Neubauer, L. G., *et al. Alcohol in blood and urine, 21.

*— Distribution of barbiturates in human body, 21.

Neuberg, C., *et al.* Identifying fructose, 263.

Neufeld, O. E., *et al.* Titrimetric detmn. of di-acidic bases (nicotine, quinine) in mixtures with mono-acidic bases, and simplified assay of nicotine, 160.

Nichol, J. C., *et al.* Identifying aryl iodides, 212.

Nicholls, J. R. Review of *Calculations of Analytical Chemistry*, 558.

— Review of *Modern Cereal Chemistry.* 4th Edn., 462.

Nierenstein, M. Obituary, 131.

Niericker, R., *et al.* Quantitative detmn. of tungsten, molybdenum, and vanadium alone and in presence of ferric iron, with 8-hydroxyquinoline, 214.

Norris, F. W., *et al. Chemical estmn. of nicotinic acid in cereals and other foods, 327.

Norris, L. C., *et al.* Chemical estmn. of riboflavine, 24.

Norton, A. R., *et al.* Separating and detmng. primary, secondary and tertiary alkaryl amines (ring-methylated and nitrogen-methylated homologues of aniline), 451.

Noyes, H. M., *et al.* Detmng. aromatic amines and substituted ureas in smokeless powder. Volumetric bromination procedure, 73.

Noyons, E. C. Iodimetric estmn. of glucose in urine and blood, 210.

O

Oertel, A. C., *et al.* Errors in spectrochemical (flame) analysis, 497.

Oesper, R. E., *et al.* Ferrous ethylenediamine sulphate as oxidimetric standard, 491.

Okell, F. L. Review of *General Chemistry.* 2nd Edn., 226.

— Review of *The Microscope, its Theory and Applications*, 557.

Oldach, C. S., *et al.* Conversion of organic sulphur to hydrogen sulphide for analysis, 115.

— Detmng. hydrogen sulphide in gases, 172.

— Identifying sulphur compounds in gas mixtures, 219.

Ormsby, A. A., *et al.* Colorimetric detmn. of pyridoxine, pyridoxal and pyridoxamine, 260.

***Osborn, G. H.** Factors affecting sodium carbonate fusion of beryl, 475.

Oschman, V. A. Separating niobium and tantalum from titanium, 373.

Ottesen, M., *et al.* Polarographic detmn. of oestrone in urine, 403.

Otvos, J. W., *et al.* Polarographic analysis of nitrite and nitrite - nitrate mixtures, 268.

P

Parks, T. D., *et al.* Amperometric titration of cyanide with silver nitrate, using rotating platinum electrode, 319.

— X-ray identification and crystallography of aldehydes and ketones as 2 : 4-dinitrophenylhydrazones, 407.

Parrack, A. L., *et al.* High-frequency oscillators in titrations and analyses, 78.

Patterson, W. I., *et al.* Detcng. and detmng. monochloroacetic acid, 405.

Pearce, D. W., *et al.* Analytical chemistry of rare earths. Active-oxygen detmn., 217.

Peden, J. D. Appointment as Deputy Public Analyst for County of Staffordshire, 397.

Pemberton, R., *et al. Microchemical detmn. of molybdenum in steel, 185.

Perlzweig, W. A., *et al.* Detmng. N^1-methylnicotinamide in urine, 363.

— Rapid fluorimetric estmn. of total pyridine nucleotides in red blood cells, 363.

Phelps, R. T., *et al.* Electron diffraction and electron microscopic study of oxide films formed on metals and alloys at moderate temperatures, 269.

— Oxide films formed on alloys at moderate temperatures. Electron diffraction and electron microscope study, 223.

Pickering, R. W., *et al.* Thiocyanate complex as means of extracting cobalt before micro-detmn. by other methods, 222.

Piguet, A., *et al.* Semi-quantitative inorganic analysis, 116.

Pinkney, E. T., *et al.* Entrainment of cobalt and sulphur in iron separations, 122.

Pitha, J. Sublimation apparatus, 126.

Pizer, N. H., *et al.* Morgan method of soil testing. IV. Spekker absorptiometer for estmng. phosphate, 124.

Pletenev, S. A., *et al.* Applying polarographic analysis to production control in non-ferrous metallurgy. I. Detmng. copper, lead and zinc impurities in metallic cadmium. II. Control of solutions and products in cobalt manufacture. III. Detmng. copper, bismuth, lead, cadmium and zinc impurities in metallic tin and lead - tin solders. IV. Detmng. impurities in metallic lead. V. Rapid detmn. of lead, cadmium and zinc in ores and refuse from ore-enrichment (zinc, lead and cadmium ores), 415–417.

Pogorelskin, M. A., *et al.* Colorimetric detmn. of DDT in milk and fatty materials, 360.

Pokrovskaja, T. L. Analysing cassiterite, 494.

Polis, B. D., *et al.* Rapid photocolorimetric microprocedure for blood sugar, 162.

Poljak, L. J. Potentiometric methods of detmng. aluminium in magnesium alloys in Elektron type; 320.

Poma, K. Polarography of mercury solutions, 552.

Porter, L. E. Semimicro detcn. of thiocyanate and halide ions, 76.

Pratt, R., *et al.* Three-hour physical development cup-plate assay for penicillin, 488.

Pregl, F. Quantitative organische Mikroanalyse. 5th Edn. (Review), 461.

***Pringle, W. J. S.** Rapid qualitative test for added iron in flour, 393.

Proffitt, P. M. C., *et al. Analysing nickel - cobalt - iron alloys used in glass-to-metal seals, 351.

*— Detmng. lead as iodate in glass analysis, 205.

Purves, C. B., *et al.* Comparative estmns. of carbonyl and carboxyl groups in chromium trioxide and hypochlorous acid oxycelluloses and oxyxylans, 72.

Putnam, S. T., *et al.* Detmng. conjugated diolefines with chloromaleic anhydride, 164.

Pyke, R., *et al.* Micro-detmn. of gaseous olefines, 418.

R

Ramasarma, G. B., *et al. Chromatographic adsorption method for estmng. provitamin-A content of foodstuffs, 194.

Rameau, J. T. L. B., *et al.* Detmng. traces of copper in vegetable material with diethyldithiocarbamate, 555.

Ramsey, L. L., *et al.* Detcng. and detmng. monochloroacetic acid, 405.

***Randles, J. E. B.** Application of cathode ray oscillograph to polarography: underlying principles, 301.

***Ransley, C. E.** Gas analysis at low pressures, 504.

Rao, S. D., *et al. Chromatographic adsorption method for estmng. provitamin-A content of foodstuffs, 194.

Rapson, W. S., *et al.* South African fish products. XXII. Periodic acid as reagent in analysis of unsaponifiable fraction of natural fats, 22.

Raventos, J. Estmng. barbituric and thiobarbituric acids in biological materials, 487.

Rees, M. W. Estmng. threonine and serine in proteins, 310.

Regnera, R. M., *et al.* Assay for choline chloride in pharmaceutical products, 23, 486.

Reimers, F. Chromatographic analysis of alkaloidal salts, 548.

— ***et al.*** Chromatographic analysis of alkaloidal salts, 548.

— Limit tests for impurities, 485.

— Limit Tests for Impurities. (Review), 500.

Reiser, R. Haemoglobin in meat scraps and tankage, 446.

Reith, J. F., *et al.* Combined micro-gravimetric and titrimetric micro-detmn. of thallium in toxicological material, 221.

— Detmng. traces of copper in vegetable material with diethyldithiocarbamate, 555.

Rendle, T., *et al.* The Nation's Food: Survey of Scientific Data. (Review), 271.

Rhodes, H. T. F. Forensic Chemistry. (Review), 127.

Ridyard, H. N. Adsorption of aneurine on sand, 68.

Rieman, W., *et al.* Detmng. boric oxide in glass, 317.

Rieman, W., third, *et al.* Kjeldahl detmn. of nitrogen. Eliminating distillation, 163.

— Quantitative separation of barium and strontium, 551.

Riesen, W. H., *et al.* Microbiological estmn. of methionine in proteins and foodstuffs, 69.

Riesenfeld, F. C., *et al.* Simultaneous detmn. of hydrogen sulphide and carbon dioxide in continuous gas stream, 495.

Riley, J. P., *et al.* Low temperature crystallisation in detmng. component acids of liquid fats. I. Fats in which oleic and linoleic acids are major components. III. Fats which contain elæostearic as well as linoleic and oleic acids, 402.

Ritchie, J. E. Appointment as Public Analyst to Counties of Aberdeen, Banff, Caithness, Kincardine, Ross and Cromarty, and Sutherland, 64.

Roach, W. A. Flame method of spectrochemical analysis, 174.

Robb, M. J. Appointment as Public Analyst to Counties of Aberdeen, Banff, Caithness, Kincardine, Ross and Cromarty, and Sutherland, 64.

Roberts, E. J., *et al.* Quantitative method for aconitic acid and aconitates, 453.

Roberts, E. R. Analysis of brass plating, 273.

Roberts, W. H. Obituary, 36, 131, 133.

Robertson, W. W., *et al.* Quantitative analysis of isomeric cresols and cresol - phenol mixtures by U.V. absorption spectra of vapours, 264.

***Robinson, F. A.** Chemistry of penicillin, 274.

Robinson, J., *et al.* Rapid fluorimetric estmn. of total pyridine nucleotides in red blood cells, 363.

Robinson, R. J., *et al.* Rapid electrometric detmn. of alkalinity of sea water, 375.

Rochat, J. Estmng. ethyl alcohol in blood, 450.

Rogers, L. B., *et al.* Behaviour of uranyl solutions in mercury reductor, 216.

Rose, J. D., *et al.* 2 : 4-Dinitrophenylsemicarbazide. Reagent for aldehydes and ketones, 212.

Rosen, F., *et al.* Rapid fluorimetric estmn. of total pyridine nucleotides in red blood cells, 363.

Rosenbaum, E. J., *et al.* Quantitative analysis of hydrocarbon mixtures by means of Raman spectra, 261.

Roth, H. Editor of Pregl's Quantitative organische Mikroanalyse. 5th Edn. (Review), 461.

Rusconi, Y., *et al.* Colorimetric detmn. of fluoride, 117.

Rutten, A. M. G. Microchemical detcn. of vanillin and bourbonal, 449.

S

Sackett, J. W., *et al.* Polarographic analysis of streptomycin, 310.

Saifer, A., *et al.* Photometric detmn. of total cholesterol in plasma or serum by modified Liebermann-Burchard reaction, 27.

Salomon, K., *et al.* Precision method for quantitative detmn. of calcium in blood plasma, 310.

Salsbury, J. M., *et al.* Micro-gravimetric detmn. of carbon monoxide, 418.

Sandell, E. B. Detmng. gallium in silicate rocks, 457.

Sandin, R. B., *et al.* Identifying aryl iodides, 212.

Sarudi, I., (v. Stetina). Separation of copper and zinc with sodium thiosulphate in analysing brass, 410.

Saur, B., *et al.* Colorimetric estmn. of proteins in body fluids, 208.

Schaeppi, Y., *et al.* Constitution of molybdenum blue, 117.

Schaltegger, H. Colorimetric estmn. of vitamin D, based on formation of carbenium salts, 70.

Schechter, M. S., *et al.* Colorimetric detmn. of DDT in milk and fatty materials, 360.

Schlenk, F., *et al.* Colorimetric detmn. of pyridoxine, pyridoxal and pyridoxamine, 260.

Schmid, K. Step-photometric detmn. of histidine, 486.

Schmidt, E. G. Detmng. sulphonamides in tungstic acid (Folin-Wu) blood filtrates, 25.

Schmidt, R. Purifying carbon rods for spectrochemical analysis. II. Carbon rods for spectrochemical analysis of solutions, 419.

Schneider, W. G., *et al.* Radioactive studies. Analytical procedure for measurement of long-lived radioactive sulphur ^{35}S, with Lauritzen electroscope, and comparison of electroscope with special Geiger counter, 80.

Schoch, E. P., *et al.* General Chemistry. 2nd Edn. (Review), 226.

Schonk, C. H. Detmng. traces of copper by extractive dithizone titration, 492.

Schuldiner, S., *et al.* Detmng. chromium by oxidation with perchloric acid, 410.

Schuster, M. C., *et al.* Prepng. powdered materials for electron microscopy, 224.

Schwarzenbach, G., *et al.* Titration methods for detmng. hardness of water, 322.

Schwed, P., *et al.* Polarographic analysis of streptomycin, 310.

Schweigert, B. S., *et al.* Hydrolysis procedures for estmng. tryptophan in proteins and foodstuffs by microbiological procedure, 25.

— Microbiological estmn. of methionine in proteins and foodstuffs, 69.

Scott, M. L., *et al.* Chemical estmn. of riboflavine, 24.

Scott-Dodd, A. Appointment as Public Analyst to Counties of Berwick, Inverness, Moray and Nairn, Orkney, Roxburgh, Selkirk and Zetland, Burghs of Edinburgh and Inverness, and small Burghs of Dunbar, North Berwick and Peebles, 64.

Scribner, B. F., *et al.* Index to the Literature on Spectrochemical Analysis, Part II, 1940 to 1945. (Review), 325.

Seaman, W., *et al.* Separating and detmng. primary, secondary and tertiary alkaryl amines (ring-methylated and nitrogen-methylated homologues of aniline), 451.

Secor, G. E., *et al.* Micro-Kjeldahl detmn. of nitrogen in gramicidin and tryptophan. Comparison of Gunning-Arnold-Dyer and Friedrich methods, 263.

Seekles, L., *et al.* Detmng. traces of copper with diethyldithiocarbamate, 555.

Shaffer, C. B., *et al.* Solid polyethylene glycols (Carbowax compounds). Quantitative detmn. in biological materials, 404.

Sheehan, J. C., *et al.* Chemical assay for penicillin G, 313.

Shepard, C. E., *et al.* Detmng. sulphathiazole and other sulpha drugs in honey, 448.

Shergold, F. A. Effect of sieve loading on results of sieve analysis of natural sands, 126.

Sherlock, A. Obituary, 423.

Sherrington, L. G., *et al.* Absorptiometric detmn. of silicon in steels, 121.

Shertinsky, E., *et al.* Microbiological estmn. of nicotinic acid based on use of *Proteus* HX19, 362.

Shrivastava, R. K., *et al. Spectrophotometric detmn. of small proportions of linolenic acid in fats, 527.

Silnitschenko, V. G., *et al.* Detmng. nickel and cobalt in iron ores with preliminary removal of iron by means of sodium fluoride, 373.

Simmons, L. M. Apparatus for detmn. boiling points, 497.

Simonsen, D. G., *et al.* Detmng. serum phosphate by molybdi-vanadate method, 312.

***Simpson, D. M.** Applications of fluorimetric analysis to study of pterins, 382.

Simpson, S. G., *et al.* Calculations of Analytical Chemistry. (Review), 558.

Slater, E. C., *et al.* Fluorimetric estmn. of riboflavine in urine, 257.

— Modification of fluorimetric method of estmng. riboflavine in biological materials, 257.

Slooff, A. Qualitative elementary analysis (of organic substances) by hydrogenation, 163.

***Smales, A. A.** Pptng. aluminium and its separation from cobalt, chromium, nickel or zinc by means of ammonium benzoate, 14.

Smiley, W. G. Precise low-pressure measurements with thermocouple gauge, 497.

Smith, C., *et al.* Estmng. penicillin in serum. Use of glucose, phenol red, and serum water, 548.

Smith, F. H. Effect of acetic acid on spectrophotometric estmn. of gossypol in aqueous alcoholic solution, 32.

Smith, G. B. L., *et al.* Solubility of magnesium ammonium phosphate hexahydrate, 118.

Smith, G. F., *et al.* Precision method for quantitative detmn. of calcium in blood plasma, 310.

Smith, H. P. Obituary, 423.

Smith, L., *et al.* Detmng. traces of iron in bacteriological media, 555.

Smith, N. D. P., *et al.* Comparison of optical and electron microscopy of pigments, 223.

Sobel, A. E., *et al.* Activated glycerol dichlorohydrin. New colorimetric reagent for vitamin A, 27.

Sobel, H., *et al.* Colorimetric estmn. of reducing steroids, 71.

Sortwell, M., *et al.* Rapid photocolorimetric microprocedure for blood sugar, 162.

Spector, N. A., *et al.* Colorimetric detmn. of traces of carbon dioxide in air, 418.

Spilsbury, Sir Bernard H. Obituary, 503.

Stace, H. C. T., *et al.* Errors in spectrochemical (flame) analysis, 497.

Stathis, E. C., *et al.* Detmng. gold with ascorbic acid, 123.

Steenberg, B., *et al.* Analysing dilute melamine resin solutions, 32.

Steigmann, A. Analytical reactions with organic reagents, 28.

Stephenson, O., *et al.* Chemical investigations of the insecticide DDT and analogues. I. Reactions of DDT and associated compounds, 218.

Stern, E. S., *et al.* Detmng. "active" hydrogen, 213.

***Stock, J. T.** Amperometric titration, 291.

*— ***et al.*** Simple direct-reading semimicrobalance, 394.

Strafford, N., *et al. Detmng. small amounts of aluminium by aurintricarboxylate method, 54.

Strain, H. H. Conditions affecting the sequence of organic compounds in Tswett adsorption columns, 77.

Strait, L. A., *et al.* Three-hour physical development cup-plate assay for penicillin, 488.

Strasburger, L. W., *et al.* Indole in shrimp, 545.

***Stuart, R. G.** Detmng. acetyl groups by trans-esterification, 235.

Sullivan, M. V., *et al.* Detmng. tetra-ethyl lead in gasoline by X-ray absorption, 269.

Sultzaberger, J. A., *et al.* Quantitative estmn. of arsenoso group, 265.

Sutton, D. A., *et al.* Simple laboratory falling-film molecular still, 174.

Swann, M. H. Volumetric detmn. of water in paints and varnishes, 170.

T

Tal, E. M., ***et al.*** Applying polarographic analysis to production control in non-ferrous metallurgy. I. Detmng. copper, lead and zinc impurities in metallic cadmium. II. Control of solutions and products in cobalt manufacture. III. Detmng. copper, bismuth, lead, cadmium and zinc impurities in metallic tin and lead - tin solders. IV. Detmng. impurities in metallic lead. V. Rapid detmn. of lead, cadmium and zinc in ores and refuse from ore-enrichment (zinc, lead and cadmium ores), 415–417.
— Polarographic detmn. of lead in lead slags, 268.
Tananaev, I. V., ***et al.*** Detmng. nickel and cobalt in iron ores with preliminary removal of iron by means of sodium fluoride, 373.
Tananaev, N. A., ***et al.*** Colorimetric detmn. of molybdenum as thiocyanate complex, 455.
Tarbell, D. S., ***et al.*** Colour reactions of unsymmetrical diarylethylenes, 489.
Tasman, A., ***et al.*** Detmng. traces of iron in bacteriological media, 555.
Taylor, R. C., ***et al.*** Molecular weight detmn. with vacuum micromanometer, 497.
— Vacuum micromanometer, 497.
Taylor, W. W. Appointment as Official Agricultural Analyst for Administrative County of the Parts of Lindsey, Lincolnshire, 254.
Teterkin, E. N. Detmng. total hardness of water by means of soap solution, 375.
Thin, R. G. Appointment as Public Analyst to Counties of Clackmannan, East Lothian (excluding Burghs of Dunbar and North Berwick), Midlothian, Peebles (excluding the Burgh of Peebles) and West Lothian, 64.
***Thistlethwaite, W. P.** Detmng. composition and constitution of ammonium phosphomolybdate and conditions affecting its pptn., 531.
Thompson, J. F., ***et al.*** Estmng. cobalt in biological materials with nitrosocresol, 113.
Thomson, R. T. Appointment as Public Analyst to Counties of Dumbarton and Stirling, and Burghs of Airdrie, Clydebank, Coatbridge, Falkirk, Hamilton, Perth, Rutherglen and Stirling, 64.
Thomson, T. Specificity of salicylaldehyde reaction for detcng. acetone, 28.
Tilleard, D. L., ***et al.*** Comparison of optical and electron microscopy of pigments, 223.
Tinsley, J., ***et al.*** Morgan method of soil testing. IV. Spekker absorptiometer for estmng. phosphate, 124.
Tošić, J., ***et al.*** Characterisation of acetic acid bacteria. I. II, 125.
Trautner, E. M., ***et al.*** Titrimetric detmn. of diacidic bases (nicotine, quinine) in mixtures with monoacidic bases, and a simplified assay of nicotine, 160.
Treadwell, W. D., ***et al.*** Constitution of molybdenum blue, 117.
— Quantitative detmn. of tungsten, molybdenum, and vanadium alone and in presence of ferric iron, with 8-hydroxyquinoline, 214.
Tristram, G. R. Applying partition chromatography to estmng. monoamino acids in proteins, 260.
Truman, R. J.,** ***et al. Detmng. tungsten in steel, using toluene-3 : 4-dithiol. Removal of molybdenum interference by selective extraction, 189.
Tschalov, N. V., ***et al.*** Detmng. low concentrations of ethyl alcohol in aqueous solutions, 313.
Tschernichov, J. A., ***et al.*** Bivalent chromium solution for volumetric detmn. of tungsten. Detmng. tungsten in tungsten concentrates and ferro-tungsten, 371.
Tzobar, L. I. Detmng. gold by back-titration, 373.

U

Ulusoy, E., ***et al.*** Isolating and identifying fatty acids as bis-(*p*-dimethylaminophenyl)-ureides, 364.
Uncles, R. F., ***et al.*** Solubility of magnesium ammonium phosphate hexahydrate, 118.
Updike, R. L., jun., ***et al.*** Prepng. standard powders for reference in particle-size measurement, 270.
***Uri, N.** Stability of cobaltous thiocyanate complex in ethyl alcohol - water mixtures and photometric detmn. of cobalt, 478.

V

Vakil, K. H. Obituary, 131.
Valov, P. Rapid qualitative and quantitative detmn. of barbiturates from post-mortem specimens, 256.
van Arkel, C. G., ***et al.*** Detmng. narceine in narceine sodium salicylate by means of Reinecke's salt, 547.
Vanas, D. W., ***et al.*** Detmng. copper in cast iron and steels with quinaldic acid, 121.
van der Kamer, J. H., ***et al.*** Detmng. specific gravity of extracts of jams, syrups, etc., based on density gradient, 399.
Van Slyke, D. D., ***et al.*** Application of Sendroy's iodimetric chloride titration to protein-containing fluids, 364.
— Detmng. carbon monoxide in blood, and total and active haemoglobin by carbon monoxide capacity, 257.
Viachirev, D. A. Polarographic analysis of brass plating baths and brass coatings, 268.
Vickerstaff, T., ***et al.*** Accuracy of colorimetric instruments in dye strength detmns., 420.
Vickery, R. C. Separating rare-earth elements by chloride volatilisation, 414.
Vigna, N. Detmng. oxygen in iron and steel, 456.
Visser, J., ***et al.*** Detmng. uranium with 8-hydroxyquinoline, 123.
Volskaja, L. P., ***et al.*** Detmng. low concentrations of ethyl alcohol in aqueous solutions, 313.
von Stetina: *see* **Sarudi.**

W

Waegemans, G., ***et al.*** Detmng. quartz content of clay fraction of soils, 217.
Wagner, R. Estmng. glycogen in whole blood and white blood cells, 162.
Wain, R. L.,** ***et al. Estmng. 2 : 2-*bis*-(*p*-chlorophenyl)-1 : 1 : 1-trichloroethane (*p,p'*-DDT) by dehydrohalogenation, 1.
Walker, T. K., ***et al.*** Characterisation of acetic acid bacteria. I. II, 125.
Wallis, T. E. Textbook of Pharmacognosy. (Review), 376.
Walters, S. L., ***et al.*** Analysis of boron trifluoride in organic liquids (ethers), 32.
— Routine analysis of sodium - potassium alloys, 317.
Warburton, G. H. Obituary, 131.

Warshowsky, B., *et al.* Analysing mixtures of mercurous and mercuric mercury and sulphuric acid, 493.
Waters, K. L., *et al.* Detmng. chlorine in 2 : 2′-dihydroxy-5 : 5′-dichlorodiphenylmethane, 31.
Waters, W. A., *et al.* Chemical investigations of the insecticide DDT and analogues. I. Reactions of DDT and associated compounds, 218.
Watkinson, S. Obituary, 131, 133.
Watt, G. W., *et al.* General Chemistry. 2nd Edn. (Review), 226.
Waugh, T. D., *et al.* Detmng. aromatic amines and substituted ureas in smokeless powder. Volumetric bromination procedure, 73.
Weir, A. B. Appointment as Public Analyst to Burgh of Aberdeen, 64.
Weisiger, J. R., *et al.* Detmng. carbon monoxide in blood, and total and active haemoglobin by carbon monoxide capacity, 257.
Wells, J. E., *et al.* Detmng. hydrogen in liquid steel, 457.
*— Microchemical detmn. of molybdenum in steel, 185.
Wendland, R. T. System of characterisation of pure hydrocarbons. Refractometric analysis as key to evaluation of structure, 114.
Wenger, P., *et al.* Colorimetric detmn. of fluoride, 117.
— Micro-separation of zinc and aluminium by means of potassium ethyl xanthogenate, 76.
— Semi-quantitative inorganic analysis, 116.
Wentworth, R. L., *et al.* Explosion in detmng. cobalt as potassium cobaltinitrite, 410.
Werbin, H., *et al.* Activated glycerol dichlorohydrin. New colorimetric reagent for vitamin A, 27.
Wertman, M., *et al.* Detmng. serum phosphate by molybdi-vanadate method, 312.
West, R. V. O., jun., *et al.* Analysing furfural-water solutions, 165.
Westover, L. M., *et al.* Detmng. serum phosphate by molybdi-vanadate method, 312.
Westwood, W., *et al. Direct colorimetric detmn. of tungsten in cast iron, 464.
Weygand, C. Organic Preparations. Translation of Part II, "Reaktionen," of *Organisch-Chemische Experimentierkunst.* (Review), 33.
White, L. M., *et al.* Micro-Kjeldahl detmn. of nitrogen in gramicidin and tryptophan. Comparison of Gunning-Arnold-Dyer and Friedrich methods, 263.
Whittle, E. G. Appointment as Official Agricultural Analyst for County Borough of Bristol, 153; and as Public Analyst, 253.
Widdowson, E. M., *et al.* Chemical Composition of Foods. 2nd Edn. (Review), 81.
Wiley, R. E., *et al.* Detmng. silicon in relatively non-volatile organosilicon compounds, 264.
Wilkes, J. B. Chromatographic detmn. of carotene, 321.
Willard, H. H., *et al.* Fractional separation of hafnium and zirconium by means of triethyl phosphate, 216.
Williams, A. L. Appointment as Public Analyst for Borough of Gosport, and Official Agricultural Analyst for County Borough of Portsmouth, 397.
Williams, K. A. Review of *Chemical Constitution of Natural Fats.* 2nd Edn., 500.
— Review of *Fatty Acids, their Chemistry and Physical Properties,* 557.
Williams, K. A. Review of *New Notation and Enumeration System for Organic Compounds,* 176.
Williams, W. L. Yeast microbiological method for estmng. nicotinic acid, 312.
Wilson, A. Appointment as Public Analyst to County of Lanark, 64.
Wilson, H. N., *et al. Detmng. nitromethane in air, 432.
*— Polarographic detmn. of tin, lead and zinc in phenol, 149.
Wilson, W. Appointment as additional Public Analyst to Counties of Dumfries, Kirkcudbright and Wigtown, and Burgh of Dumfries, 64.
Winslow, E. H., *et al.* Traces of oxygen in hydrogen or nitrogen, 267.
Witte, A. H. Identifying sulphonamides, 546.
***Wokes, F.** Oxalic acid extractant in vitamin C assay, 63.
*— ***et al.*** Chemical estmn. of nicotinic acid in cereals and other foods, 327.
Wolfrom, M. L. Chromatography of sugars and their derivatives, 316.
Wood, C. E., *et al.* Detmng. rates of de-oxidation of iron oxide materials in reducing gases, 318.
***Wood, E. C.** Computation of microbiological assays of amino acids and other growth factors, 84.
— Review of *Industrial Experimentation.* 2nd Edn., 422.
Wood, S., *et al.* Hydrogen-peroxide-treated peptone in media for microbiological detmn. of amino acids, 70.
— Microbiological estmn. of amino acids. III. Methionine, 209.
Woods, J. T., *et al.* Separating and detmng. primary, secondary and tertiary alkaryl amines (ring-methylated and nitrogen-methylated homologues of aniline), 451.
Wordsworth, C. H. Appointment as Public Analyst for Borough of Luton, 152.
Wredden, J. H. The Microscope, its Theory and Applications. (Review), 557.
Wright, E. R., *et al.* Separating calcium from magnesium by oxalate method in samples of high magnesium - calcium ratio, 118.
Wyatt, G. H. Review of *Quantitative organische Mikroanalyse.* 5th Edn., 461.
*— ***et al.*** Photometric detmn. of dissolved oxygen in condensates and feed waters by means of starch-iodide complex, 340.
Wyatt, P. F., *et al. Detmng. small amounts of aluminium by aurintricarboxylate method, 54.
Wyler, J. A. Detmng. dipenta-erythritol in mixtures of penta-erythritol and dipenta-erythritol, 365.
***Wylie, A. W.** Detmng. sulphate in sodium dichromate, 250.

Y

Yoe, J. H., *et al.* Colorimetric detmn. of titanium with disodium-1 : 2-dihydroxybenzene-3 : 5-disulphonate, 552.
— Micro-gravimetric detmn. of carbon monoxide, 418.
Young, R. S., *et al.* Entrainment of cobalt and sulphur in iron separations, 122.
Young, W. S., *et al.* Molecular weight detmn. with vacuum micromanometer, 497.
— Vacuum micromanometer, 497.

INDEX TO SUBJECTS

* Denotes original papers and notes published in *The Analyst.*

A

Acetic acid bacteria: Characterisation of ——. I. II. Tošić and Walker, 125.
Effect of —— on spectrophotometric estmn. of gossypol in aqueous alcoholic solution. Smith, 32.
***Acetone:** Estmng. volatile matter content of propellant explosives. 3. Estmng. ——. Bonner, 434.
Solubility of acetylene in ——. Brameld and Clark, 31.
Specificity of salicylaldehyde reaction for detcng. ——. Thomson, 28.
***Acetyl groups:** Detmng. —— by trans-esterification. Stuart, 235.
Acetylene: Solubility of —— in acetone. Brameld and Clark, 31.
Acid(s): Chromatographic adsorption of amino —— on organic exchange resins. Cleaver, Hardy and Cassidy, 450.
Detmng. and isolating organic —— in fruit. Isherwood, 261.
Detmng. free —— in the presence of aluminium salts. Graham, 122.
Detmng. free fatty —— in dried egg powders. Johnson and Kline, 67.
Fatty —— of corn (maize) oil. Baur, jun., and Brown, 448.
Fatty ——, their Chemistry and Physical Properties. Markley. (Review), 557.
Isolating and identifying fatty —— as bis-(*p*-dimethylaminophenyl)-ureides. Breusch and Ulusoy, 364.
Low-temperature crystallisation in detmng. component —— of liquid fats. I. Fats in which oleic and linoleic —— are major components. Hilditch and Riley. II. Fats which contain linolenic as well as linoleic and oleic ——. Gunstone and Hilditch. III. Fats which contain elæostearic as well as linoleic and oleic ——. Hilditch and Riley, 402.
Micro-titration of organic ——. Ingold, 114.
New specific colour reaction of hexuronic ——. Dische, 365.
Aconitates: Quantitative method for aconitic acid and ——. Roberts and Ambler, 453.
Aconitic acid: Ditto, ditto. Roberts and Ambler, 453.
Air: Colorimetric detmn. of carbon dioxide in ——. Spector and Dodge, 418.
Oxidation of metallic hydroxides by current of ——. Fireman, 214.
Alcohol: Detmng. low concentrations of ethyl —— in aqueous solutions. Tschalov and Volskaja, 313.
Estmng. ethyl —— in blood. Rochat, 450.
*in blood and urine. Andrew and Neubauer, 21.
Aldehydes: 2 : 4-Dinitrophenylsemicarbazide. Reagent for —— and ketones. McVeigh and Rose, 212.
X-ray identification and crystallography of —— and ketones as 2 : 4-dinitrophenylhydrazones. Clark, Kaye and Parks, 407.
Alkaline earth metals: Detcng. —— in cell tissue of plants. Haller, 71.
Alkalinity: Rapid electrometric detmn. of —— of sea water. Anderson and Robinson, 375.
Alkaloidal salts: Chromatographic analysis of ——. Reimers and Gottlieb, 548.
Alkaloids: Polarographic study of ——. V. Kirkpatrick, 409.
Alkyl halides: 6-Nitro-2-mercaptobenzothiazole ("Nitro-Captax") as reagent for identifying ——. Cutter and Golden, 550.
Alloxan: Detmng. ——. Brückmann, 24.
***Alloying elements:** Spot-tests for detcng. —— in lead-base alloys. Evans and Higgs, 105.
*Spot-tests for detcng. —— in tin-base alloys. Evans and Higgs, 439.
*Spot-tests for detcng. —— in zinc-base alloys. Evans and Higgs, 101.
***Alloys:** Analysing nickel - cobalt - iron alloys used in glass-to-metal seals. Chirnside, Cluley and Proffitt, 351.
Detmng. nickel in cobalt steels and ——. Kirtchik, 553.
Electron diffraction and electron microscopic study of oxide films formed on metals and —— at moderate temperatures. Phelps, Gulbransen and Hickman, 269.
Oxide films formed on —— at moderate temperatures. Electron diffraction and electron microscope study. Gulbransen, Phelps and Hickman, 223.
Potentiometric detmn. of aluminium in magnesium —— of Elektron type. Poljak, 320.
Routine analysis of sodium - potassium ——. Walters and Miller, 317.
*Spot-tests for detcng. alloying elements in lead-base ——. Evans and Higgs, 105.
*Spot-tests for detcng. alloying elements in tin-base ——. Evans and Higgs, 439.
*Spot-tests for detcng. alloying elements in zinc-base ——. Evans and Higgs, 101.
Aluminium: Detmng. —— in high alloy steel. Fogelson and Kalmikova, 493.
*Detmng. small amounts of —— by aurintricarboxylate method. Strafford and Wyatt, 54.
Micro-separation of zinc and —— by means of potassium ethyl xanthogenate. Wenger, Abramson and Besso, 76.
Potentiometric detmn. of —— in magnesium alloys of Elektron type. Poljak, 320.
*Pptng. —— and separating it from cobalt, chromium, nickel or zinc by means of ammonium benzoate. Smales, 14.
Aluminium oxide: Tests for —— for chromatographic analysis. Christensen, 556.
Aluminium salts: Detmng. free acid in the presence of ——. Graham, 122.
American Oil Chemists' Soc.: Official and Tentative Methods of ——. 2nd Edn. (Notice), 157.
Amine(s): Detmng. aromatic —— and substituted ureas in smokeless powder. Volumetric bromination procedure. Waugh, Harbottle and Noyes, 73.
Identifying acidic oxycellulose by means of aromatic —— and diamines. Krajčinović and Krajčinović, 366.
*oxide group: Reductometric detmn. of sulphoxide group and ——. Glynn, 248.
Separating and detmng. primary, secondary and tertiary alkaryl —— (ring-methylated and nitrogen-methylated homologues of aniline). Seaman, Norton, Woods and Bank, 451.

Amino acids: Application of partition chromatography to estmng. mono- —— in proteins. Tristram, 260.

  Chromatographic adsorption of —— on organic exchange resins. Cleaver, Hardy and Cassidy, 450.

  Chromatography in separating and detmng. basic ——. Bergdoll and Doty, 77.

  *Computation of microbiological assays of —— and other growth factors. Wood, 84.

  Hydrogen-peroxide-treated peptone in media for microbiological detmn. of ——. Lyman, Moseley, Wood and Hale, 70.

  Microbiological estmn. of ——. III. Methionine. Lyman, Moseley, Butler, Wood and Hale, 209.

Ammonia: Improved Kjeldahl trap and —— distillation apparatus. Lovecy, 420.

***Ammonium benzoate:** Pptng. aluminium and separating it from cobalt, chromium, nickel or zinc by means of ——. Smales, 14.

  *System of qualitative analysis for common metals in presence of phosphates, using ——. Austin, 443.

***Ammonium phosphomolybdate:** Detmng. composition and constitution of —— and conditions affecting its pptn. Thistlethwaite, 531.

***Amperometric titration.** Stock, 291.

Anaesthetic: Effect of the —— and rate of injection of digitalis upon lethal dose in cats. Holck, Kimura and Bartels, 449.

***Analysis:** Apparatus for gas —— by soap film method. Gooderham, 520.

  *Applications of electrometric methods in ——. Carter, 94.

  *Applications of fluorimetric —— to study of pterins. Simpson, 382.

  Conversion of organic sulphur to hydrogen sulphide for ——. Field and Oldach, 115.

  Flame method of spectrochemical ——. Roach, 174.

  Four papers on fluorimetric ——. 377.

  Index to the Literature on Spectrochemical ——. Part II, 1940 to 1945. Scribner and Meggers. (Review), 325.

  Method of semi-quantitative inorganic ——. Wenger, Monnier and Piguet, 116.

  Most economical sampling for chemical ——. Churchman, 323.

  *of hydrocarbon gases by low temperature distillation. Hooper, 513.

  *Procedure for gas —— at low pressures. Ransley, 504.

  Qualitative elementary —— of organic substances by hydrogenation. Slooff, 163.

  Standardised Substances for Spectrography, Chemical —— and Research. Johnson, Matthey and Co., Ltd., 254.

  *System of qualitative —— for common metals in presence of phosphates, using ammonium benzoate. Austin, 443.

  three papers on electrometric ——. 90.

Analytical Chemistry: Calculations of ——. Hamilton and Simpson. (Review), 558.

Analytical control methods: Improving precision by repeated measurements. Application to ——. Mandel, 324.

Analytical reactions with organic reagents. Steigmann, 28.

Aneurine: Adsorption of —— on sand. Ridyard, 68.

Aniline: Separating and detmng. primary, secondary and tertiary alkaryl amines (ring-methylated and nitrogen-methylated homologues of ——). Seaman, Norton, Woods and Bank, 451.

Animal: Maintenance of laboratory —— colonies. New York Academy of Sciences, 501.

Antimony: Colorimetric detmn. of ——. Kokorin, 319.

Apparatus for detmng. boiling points. Simmons, 497.

  *for gas analysis by soap film method. Gooderham, 520.

  Improved Kjeldahl trap and ammonia distillation ——. Lovecy, 420.

  *Recent developments in —— for pH measurements and electro-titrations. Lauchlan, 90.

  Simple sublimation ——. Pitha, 126.

  Supply of laboratory —— and chemicals. 176, 227.

Appointments, Official. 64, 152, 153, 253, 254, 397, 543.

Aromatic content: Picric acid method of detmng. —— of aviation gasoline. Gambrill and Martin, 73.

***Arsenic:** Detmng. copper and —— in iron pyrites by internal electrolysis. Davies and Key, 17.

  Radioactive studies. Analytical procedure for measurement of radioactive —— of 90-day half-life. Henriques and Margnetti, 80.

  Source of error in Gutzeit method for ——. Goldstone, 159.

Arsenious acid: Volumetric detmn. of mixtures of ferrocyanide and ——. Lang, 551.

Arsenoso group: Quantitative estmn. of ——. Banks and Sultzaberger, 265.

Aryl iodides: Identifying ——. Nichol and Sandin, 212.

Ascorbic acid: Detmng. gold with ——. Stathis and Gatos, 123.

Ash: Rapid semi-micro volumetric detmn. of potash in leaf ——. Chapman, 374.

***Assays:** Computation of microbiological —— of amino acids and other growth factors. Wood, 84.

***Atmosphere:** Estmng. mercury compounds in ——. Milton and Duffield, 11.

***Aurintricarboxylate** method: Detmng. small amounts of aluminium by ——. Strafford and Wyatt, 54.

Austenitic chromium - nickel steels: Detmng. phosphorus in ——. Methods of analysis of Metallurgy Division of B.I.S.R.A., 412.

***Azo dyes:** Colour reaction of certain classes of —— with copper. Alexander, 56.

B

Bacteria: Characterisation of acetic acid ——. I. II. Tošić and Walker, 125.

Bacteriological media: Detmng. traces of iron in ——. Tasman and Smith, 555.

Bacteriostatics: Estmng. penicillin and other ——. Fielding, 256.

***Balance:** Simple direct-reading semi-micro ——. Stock and Fill, 394.

***Barbiturates:** Distribution of —— in the human body. Andrew and Neubauer, 21.

  Rapid qualitative and quantitative detmn. of —— from post-mortem specimens. Valov, 256.

Barbituric acid: Estmng. —— and thiobarbituric acid in biological materials. Raventos, 487.

Barium: Quantitative separation of —— from strontium. Beyer and Rieman, 551.

Bases: Titrimetric detmn. of di-acidic —— (nicotine, quinine) in mixtures with mono-acidic ——, and simplified assay of nicotine. Trautner and Neufeld, 160.

***Beakers:** Evaporation from —— with radiant heater. Howes, 111.

***Benzene hexachloride:** Detmng. small amounts of hexachloro*cyclo*hexane (——). Howard, 427.

Benzine: Phase-volumetric analysis of mixtures of —— and aromatic hydrocarbons. de Goeij, 549.

***Beryl:** Factors affecting sodium carbonate fusion of ——. Osborn, 475.

***Bio-assay:** Statistical background in ——. Fieller, 37.

Biological fluids: Simplified detmn. of sodium in ——. Bradbury, 112.

Biological materials: Estmng. cobalt in —— with nitrosocresol. Ellis and Thompson, 113.
  Modification of fluorimetric method of estmng. riboflavine in ——. Slater and Morell, 257.
  Polarographic detmn. of tin in foods and ——. Godar and Alexander, 111.
  Solid polyethylene glycols (Carbowax compounds). Quantitative detmn. in ——. Shaffer and Critchfield, 404.

1 : 2-Bis(*p*′-chlorophenyl) ethane: Detmng. —— in DDT dusts and oil solutions. LaClair, 170.

***2 : 2-Bis-(*p*-chlorophenyl)-1 : 1 : 1-trichloroethane:** Estmng. —— by methods depending on dehydrohalogenation. Wain and Martin, 1.

Bis-(*p*-Dimethylaminophenyl)-ureides: Isolating and identifying fatty acids as ——. Breusch and Ulusoy, 364.

Bismuth: Applying polarographic analysis to production control in non-ferrous metallurgy. III. Detmng. copper, ——, lead, cadmium and zinc impurities in metallic tin and lead-tin solders. Pletenev, Arefyeva, Tal and Dubovitzkaja, 416.
  Photometric detmn. of —— in copper. Kokorin and Dermanova, 265.

Bisulphite method: Micro-detmn. of pentoses by ——. Heines, 267.

***Blood:** Alcohol in —— and urine. Andrew and Neubauer, 21.
  Detmng. carbon monoxide in —— and total and active haemoglobin by carbon monoxide capacity. van Slyke, Hiller, Weisiger and Cruz, 257.
  Estmng. ethyl alcohol in ——. Rochat, 450.
  Estmng. glycogen in whole —— and white —— cells. Wagner, 162.
  filtrates: Detmng. sulphonamides in tungstic acid (Folin-Wu) ——. Schmidt, 25.
  Iodimetric estmn. of glucose in urine and ——. Noyons, 210.
  Microbiological estmn. of riboflavine in ——. Bradford and Coke, 24.
  plasma: Precision method for quantitative detmn. of calcium in ——. Salomon, Gabrio and Smith, 310.
  Rapid fluorimetric estmn. of total pyridine nucleotides in red —— cells. Levitas, Robinson, Rosen, Huff and Perlzweig, 363.
  serum: Estmng. vitamin A and carotene in small quantities of ——. Bessey, Lowry, Brock and Lopez, 161.
  sugar: Rapid photocolorimetric micro-procedure for ——. Polis and Sortwell, 162.

***Body:** Distribution of barbiturates in the human ——. Andrew and Neubauer, 21.

Boiler(s): Analysing external deposits from ——. I. Nature of —— deposits and their examination. Crossley and Edwards. II. Analysing deposits rich in phosphates. Edwards. III. Analysing water-soluble fraction of the deposits Edwards and Flint, 119.

Boiling points: Apparatus for detmng. ——. Simmons, 497.

Book Reviews:
  Bacharach and Rendle. The Nation's Food, 271.
  Brownlee. Industrial Experimentation. 2nd Edn., 422.
  Dorée. Methods of Cellulose Chemistry, 422.
  Dyson. New Notation and Enumeration System for Organic Compounds, 176.
  Hamilton and Simpson. Calculations of Analytical Chemistry, 558.
  Hilditch. Chemical Constitution of Natural Fats. 2nd Edn., 500.
  Karrer. Organic Chemistry. 3rd English Edn., 421.
  Kent-Jones and Amos. Modern Cereal Chemistry, 462.
  McCance and Widdowson. Chemical Composition of Foods, 81.
  Markley. Fatty Acids, their Chemistry and Physical Properties, 557.
  Maxwell. Current Waterworks Practice, 270.
  Pregl. Quantitative organische Mikroanalyse, 461.
  Reimers and Gottlieb. Limit Tests for Impurities, 500.
  Rhodes. Forensic Chemistry. 2nd Edn., 127.
  Schoch, Felsing and Watt. General Chemistry. 2nd Edn., 226.
  Scribner and Meggers. Index to the Literature on Spectrochemical Analysis, Part II, 1940 to 1945. 325.
  Wallis. Textbook of Pharmacognosy, 376.
  Weygand. Organic Preparations, 33.
  Wredden. The Microscope, Theory and Applications, 557.
  Penicillin: Its Properties, Uses and Preparations, 225.
  Pharmacopoeia of the United States of America. 13th Revision, 498.
  Thorpe's Dictionary of Applied Chemistry. 4th Edn. Vol. VIII. Meth - Oils, Essential, 499.

Boric oxide: Detmng. —— in glass. Hollander and Rieman, 317.

Boron: Spectro-analytical detmn. of —— in steel. Gegetschkori and Falkova, 411.

Boron trifluoride: Analysing —— in organic liquids (ethers). Walters and Miller, 32.

Bourbonal: Microchemical detcn. of vanillin and ——. Rutten, 449.

Brass: Colorimetric detmn. of iron in —— and bronze. Goodman, 494.
  plating: Analysing ——. Roberts, 273.
  Polarographic analysis of —— plating baths and —— coatings. Viachirev, 268.
  Separating copper and zinc with sodium thiosulphate in analysing ——. Sarudi (v. Stetina), 410.

Bread (Control and Max. Prices) Order, 1947. 398.

British Iron and Steel Research Association: Detmng. phosphorus in austenitic chromium-nickel steels. Methods of analysis of Metallurgy Division of ——, 412.

Bronze: Colorimetric detmn. of iron in brass and ——. Goodman, 494.

Butacaine sulphate: Microscopical identification of —— (Butyn) and procaine hydrochloride (Novocaine). Keenan, 547.

Butyn: Ditto, ditto. Keenan, 547.

C

Cacotheline in volumetric analysis. I. Detmng. calcium salts by titration with sodium oxalate. II. Detmng. tervalent iron by titration with stannous chloride solution. Kutschment and Gengrinovitsch, 492.

Cadmium: Applying polarographic analysis to production control in non-ferrous metallurgy. I. Detmng. copper, lead and zinc impurities in metallic ——. Pletenev, Arefyeva, Tal and Dubovitzkaja, 415.
  Ditto. III. Detmng. copper, bismuth, lead, —— and zinc impurities in metallic tin and lead-tin solders. Pletenev, Arefyeva, Tal and Dubovitzkaja, 416.
  Ditto. V. Rapid detmn. of lead, —— and zinc in ores and refuse from ore-enrichment (zinc, lead and —— ores). Pletenev, Arefyeva, Tal and Dubovitzkaja, 416.
  Silver sulphide method for detmng. ——. Lapin, 373.

Caesalpinioideae: Seed oils of some Sudan ——. Grindley, 111.

Calcium: Detmng. —— in magnesite and fused magnesia. Hazel and Eglof, 168.
  Precision method for quantitative detmn. of —— in blood plasma. Salomon, Gabrio and Smith, 310.
  Semi-micro detmn. of —— in foodstuffs. Kniphorst, 159.
  Separating —— from magnesium by oxalate method in samples of high magnesium - —— ratio. Wright and Delaune, 118.

***Calcium oxide:** Detmng. —— in low-lime refractory magnesia. Gilbert, 482.

Calcium salts: Cacotheline in volumetric analysis. I. Detmng. —— by titration with sodium oxalate. Kutschment and Gengrinovitsch, 492.

***Calcium tartrate:** Formation of racemic —— in wines. Cambitzi, 542.

Calculations of Analytical Chemistry. Hamilton and Simpson. (Review), 558.

Canavanine: Colorimetric estmn. of ——. Archibald, 25.

Canned Fruit and Vegetables (Amendment No. 2) Order, 1947. 543.

Canned Meat: Meat Products, Canned Soup and —— (Control and Max. Prices) (Amendment No. 3) Order, 1946. 64.
  Meat Products and —— (Control and Max. Prices) Order, 1947. 111; (Amendment) Order, 1947. 153.

Canned Soup: Meat Products, —— and Canned Meat (Control and Max. Prices) (Amendment No. 3) Order, 1946. 64.

Canned Vegetables: Canned Fruit and —— (Amendment No. 2) Order, 1947. 543.

Capillarimeter: Precision ——. Jasper and Herrington, 460.

Carbenium salts: Colorimetric estmn. of vitamin D, based on formation of ——. Schaltegger, 70.

Carbon ^{14}C: Radioactive studies. Analytical method for detmng. long-life ——. Henriques and Margnetti, 80.
  rods: Purifying —— for spectrochemical analysis. I. Simple apparatus for purifying by resistance heating. Hoogland. II. —— for spectrochemical analysis of solutions. Schmidt. III. Deinum, 419.

Carbon dioxide: Colorimetric detmn. of traces of —— in air. Spector and Dodge, 418.

Carbon dioxide—*continued*
  Simultaneous detmn. of hydrogen sulphide and —— in continuous gas stream. Blohm and Riesenfeld, 495.

Carbon monoxide: Detmng. —— in blood and total and active haemoglobin by —— capacity. van Slyke, Hiller, Weisiger and Cruz, 257.
  Micro-gravimetric detmn. of ——. Salsbury, Cole and Yoe, 418.

Carbonyl groups: Comparative estmns. of —— and carboxyl groups in chromium trioxide and hypochlorous acid oxycelluloses and oxyxylans. Meesook and Purves, 72.

Carbowax compounds: Solid polyethylene glycols (——). Quantitative detmn. in biological materials. Shaffer and Critchfield, 404.

Carboxyl groups: Comparative estmns. of carbonyl and —— in chromium trioxide and hypochlorous acid oxycelluloses and oxyxylans. Meesook and Purves, 72.

Carotene: Chromatographic detmn. of ——. Wilkes, 321.
  *Detmng. —— in dried grass. Nelson, 200.
  Estmng. vitamin A and —— in small quantities of blood serum. Bessey, Lowry, Brock and Lopez, 161.

Cassia oil: Chemical microscopy of essential oils. IV. —— and cinnamon oil. Greene, 489.

Cassiterite: Analysing ——. Pokrovskaja, 494.

Cast iron: Detmng. copper in —— and steels with quinaldic acid. Flagg and Vanas, 121.
  *Direct colorimetric detmn. of tungsten in ——. Westwood and Mayer, 464.
  Pyrophosphoric acid in detmng. nickel in —— and steel. Kononiuk, 413.

Cats: Effect of the anaesthetic and rate of injection of digitalis upon its lethal dose in ——. Holck, Kimura and Bartels, 449.

***Cathode ray oscillograph:** Applying —— to polarography: general lay-out and uses of the polarograph. Airey, 304.
  *Applying —— to polarography: underlying principles. Randles, 301.

***Caustic soda:** Detmng. small quantities of manganese in ——. Ethrington and Hughes, 472.

Cell tissue: Detcng. alkaline earth metals in —— of plants. Haller, 71.

Cellulose in wood. T.A.P.P.I. Tentative Standard T17 m-46. 366.
  Methods of —— Chemistry. Dorée. 2nd Edn. (Review), 422.

***Cereal(s):** Chemical estmn. of nicotinic acid in —— and other foods. James, Norris and Wokes, 327.
  Modern —— Chemistry. Kent-Jones and Amos. 4th Edn. (Review), 462.

Cerium: New principle of fractionation of rare earths with nitrilotriacetate. Separating lanthanum and ——. Beck, 74.
  Two new selective —— reactions. Herzfeld, 414.

***Chapman and McFarlane method** for estmng. reducing groups in milk powder. Lea, 336.

Characterisation of acetic acid bacteria. I. II. Tošić and Walker, 125.
  of pure hydrocarbons. Refractometric analysis as key to evaluation of structure. Wendland, 114.

Cheeses: Modified Scharer phosphatase test for milk and soft ——. Horwitz, 544.

Chemical Composition of Foods. McCance and Widdowson. 2nd Edn. (Review), 81.

Chemical Society: Presentation of Address from Society of Public Analysts, 327.

Chemicals: Supply of laboratory apparatus and ——, 176, 227, 326, 376.
Chemistry: Forensic ——. Rhodes. 2nd Edn. (Review), 127.
  General ——. Schoch, Felsing and Watt. 2nd Edn. (Review), 226.
  Organic ——. Karrer. 3rd English Edn. (Review), 421.
  Thorpe's Dictionary of Applied ——. 4th Edn. Vol. VIII. Meth - Oils, Essential. (Review), 499.
Chicken manure: Estmng. —— in egg products from uric acid content. Duggan, 400.
Chloride(s): Applying Sendroy's iodimetric —— titration to protein-containing fluids. Van Slyke and Hiller, 364.
  Micro-detmn. of —— by means of mercurous iodate. Avaliani, 370.
  volatilisation : Separating rare-earth elements by ——. Vickery, 414.
Chlorine: Detmng. available —— in paper. Birchard, 493.
  Detmng. —— in 2 : 2′-dihydroxy-5 : 5′-dichlorodiphenylmethane. Jenkins, Waters and Beal, 31.
  Detmng. DDT in milk by detmng. organic ——. Carter, 361.
Chloromaleic anhydride: Detmng. conjugated diolefines with ——. Putnam, Moss and Hall, 164.
Cholesterol: Photometric detmn. of total —— in plasma or serum by modified Liebermann-Burchard reaction. Saifer and Kammerer, 27.
Cholesteryl linoleate: Detmng. linoleic acid in ——. Front and Daubert, 314.
Choline chloride: Assay for —— in pharmaceutical products. Gakenheimer and Regnera, 23, 486.
Chromatographic adsorption of amino acids on organic exchange resins. Cleaver, Hardy and Cassidy, 450.
Chromatographic analysis: Tests for aluminium oxide for ——. Christensen, 556.
Chromatographic resolution of quinone oximes. Gullstrom, Burchfield and Judy, 77.
Chromatographic spot tests. Hopf, 556.
Chromatography: Application of partition —— to estmng. monoamino acids in proteins. Tristram, 260.
  in separating and detmng. basic amino acids. Bergdoll and Doty, 77.
  of sugars and their derivatives. Georges, Bower and Wolfrom, 316.
Chrome liquors: Ion exchange resins in study of ——. Adams, 169.
Chromium: Bivalent —— solution for volumetric detmn. of tungsten. Detmng. tungsten in tungsten concentrates and ferro-tungsten. Tschernichov and Gorjuschina, 371.
  Detmng. —— by oxidation with perchloric acid. Schuldiner and Clardy, 410.
  Detmng. phosphorus in austenitic —— - nickel steels. Methods of analysis of Metallurgy Division of B.I.S.R.A., 412.
  *Pptng. aluminium and separating it from cobalt, ——, nickel or zinc by means of ammonium benzoate. Smales, 14.
  target : Identifying organic compounds by —— X-ray diffraction powder patterns. Matthews and Michell, 174.
Chromium trioxide: Comparative estmns. of carbonyl and carboxyl groups in —— and hypochlorous acid oxycelluloses and oxyxylans. Meesook and Purves, 72.
  *test for cocaine hydrochloride. Henry and Mansour, 253.
Cinnamon oil: Chemical microscopy of essential oils. IV. Cassia oil and ——. Greene, 489.
Citrate(s): Metallic complexes of tartrates and —— ; structure and behaviour in dilute solutions. I. Cupric and nickelous complexes. Bobtelsky and Jordan, 213.
  Polarographic investigations of oxalate, ——, and tartrate complexes of ferric and ferrous iron. Lingane, 370.
Clay(s): Detmng. quartz content of —— fraction of soils. de Leenheer and Waegemans, 217.
  Identifying and estmng. montmorillonite group of minerals, with special reference to soil ——. MacEwan, 218.
***Cobalt:** Analysing nickel - —— - iron alloys used in glass-to-metal seals. Chirnside, Cluley and Proffitt, 351.
  Applying polarographic analysis to production control in non-ferrous metallurgy. II. Control of solutions and products in —— manufacture. Pletenev, Arefyeva, Tal and Dubovitzkaja, 415.
  Detmng. iron in presence of ——. Brown, 122.
  Detmng. nickel and —— in iron ores with preliminary removal of iron by means of sodium fluoride. Tananaev and Silnitschenko, 373.
  Entrainment of —— and sulphur in iron separations. Pinkney, Dick and Young, 122.
  Estmng. —— in biological materials with nitrosocresol. Ellis and Thompson, 113.
  Explosion in detmng. —— as potassium cobaltinitrite. Broughton, Laing and Wentworth, 410.
  *Pptng. aluminium and separating it from ——, chromium, nickel or zinc by means of ammonium benzoate. Smales, 14.
  *Stability of cobaltous thiocyanate complex in ethyl alcohol - water mixtures and photometric detmn. of ——. Uri, 478.
  Thiocyanate complex as means of extracting —— before micro-detmn. by other methods. Bayliss and Pickering, 222.
Cobalt alloys: Detmng. nickel in cobalt steels and ——. Kirtchik, 553.
Cobalt steels: Ditto, ditto. Kirtchik, 553.
***Cobaltous thiocyanate:** Stability of —— complex in ethyl alcohol - water mixtures and photometric detmn. of cobalt. Uri, 478.
***Cocaine hydrochloride:** Chromium trioxide test for ——. Henry and Mansour, 253.
Colorimetric instruments: Accuracy of —— in dye strength detmns. Davies, Giles and Vickerstaff, 420.
***Combustion:** Oxidation of nitrogen during micro- —— of organic substances. Heron, 142.
Complexes: Metallic —— of tartrates and citrates ; structure and behaviour in dilute solutions. I. Cupric and nickelous complexes. Bobtelsky and Jordan, 213.
***Condensates:** Photometric detmn. of dissolved oxygen in —— and feed waters by means of starch-iodide complex. Bairstow, Francis and Wyatt, 340.
Constancy: Factors affecting —— of analytical weights. Craig, 420.
Control methods: Improving precision by repeated measurements. Application to analytical ——. Mandel, 324.
Copper: Applying polarographic analysis to production control in non-ferrous metallurgy. I. Detmng. ——, lead and zinc impurities in metallic cadmium. Pletenev, Arefyeva, Tal and Dubovitzkaja, 415.

Copper—*continued*
Ditto. III. Detmng. ——, bismuth, lead, cadmium and zinc impurities in metallic tin and in lead-tin solders. Pletenev, Arefyeva, Tal and Dubovitzkaja, 416.
*Colour reaction of certain classes of azo dyes with ——. Alexander, 56.
*Detmng. —— and arsenic in iron pyrites by internal electrolysis. Davies and Key, 17.
Detmng. —— in cast iron and steels with quinaldic acid. Flagg and Vanas, 121.
*Detmng. —— in food. High, 60.
Detmng. traces of —— by extractive dithizone titration. Schonk, 492.
Detmng. traces of —— in vegetable matter with diethyldithiocarbamate. Reith, Rameau, Mulder, Seekles, Kniphorst and Deijs, 555.
Photometric detmn. of bismuth in ——. Kokorin and Dermanova, 265.
Separating —— and zinc with sodium thiosulphate in analysing brass. Sarudi (v. Stetina), 410.

Corn: Fatty acids of —— (maize) oil. Baur, jun., and Brown, 448.

Cresol(s): Quantitative analysis of isomeric —— and —— -phenol mixtures by U.V. absorption spectra of vapours. Robertson, Ginsburg and Matsen, 264.

Cryolite: Rapid detmn. of sulphate impurities in ——. Karjakin and Muraschova, 266.

Crystallisation: Low-temperature —— in detmng. component acids of liquid fats. I. Fats in which oleic and linoleic acids are major components. Hilditch and Riley. II. Fats which contain linolenic as well as linoleic and oleic acids. Gunstone and Hilditch. III. Fats which contain elaeostearic as well as linoleic and oleic acids. Hilditch and Riley, 402.

Crystallography: X-ray identification and —— of aldehydes and ketones as 2 : 4-dinitrophenylhydrazones. Clark, Kaye and Parks, 407.

***Crystals:** Separation of —— and "gums" on micro or semimicro scale. Bacharach, 244.

Cupric complexes: Metallic complexes of tartrates and citrates; structure and behaviour in dilute solutions. I. —— and nickelous complexes. Bobtelsky and Jordan, 213.

Cyanamide: Detmng. —— derivatives in complex mixtures. Korinfski, 368.
Qualitative analysis of mixtures of —— derivatives. Korinfski, 367.

Cyanide: Amperometric titration of —— with silver nitrate, using rotating platinum electrode. Laitinen, Jennings and Parks, 319.

***Cyclohexane:** Prepng. —— for spectroscopic work. Ashmore, 206.

D

Dairy products: Fat detmn. in ——. Janse, 485.

DDD: Detmng. DDT in presence of ——. Claborn, 554.

DDT: Chemical investigations of the insecticide —— and its analogues. I. Reactions of —— and associated compounds. Forrest, Stephenson and Waters, 218.
Colorimetric detmn. of —— in milk and fatty materials. Schechter, Pogorelskin and Haller, 360.
Detmng. 1 : 2-*bis*(*p*′-chlorophenyl) ethane in —— dusts and oil solutions. LaClair, 170.
Detmng. —— deposits on fruits, vegetables and vegetation. Carter and Hubanks, 401.

DDT—*continued*
Detmng. —— in milk by detmng. organic chlorine. Carter, 361.
Detmng. —— in presence of DDD. Claborn, 554.

******p,p*′**-DDT:** Estmng. 2 : 2-bis-(*p*-chlorophenyl)-1 : 1 : 1-trichloroethane (——) by methods depending on its dehydrohalogenation. Wain and Martin, 1.

Density: Application of differential manometer to measurement of —— and specific gravity with special reference to temperature effects. Cowherd, 80.
gradient: Detmng. specific gravity of extracts of jams, syrups, etc., based on ——. van der Kamer and Jansen, 399.

De-oxidation: Detmng. rates of —— of iron oxide materials in reducing gases. Barrett and Wood, 318.

Deposits: Analysis of external —— from boilers. I. Nature of boiler —— and their examination. Crossley and Edwards. II. Analysing —— rich in phosphates. Edwards. III. Analysing water-soluble fraction of the ——. Edwards and Flint, 119.

Diamines: Identifying acidic oxycellulose by means of aromatic amines and ——. Krajčinović and Krajčinović, 366.

Diarylethylenes: Colour reactions of unsymmetrical ——. Tarbell and Lindstrom, 489.

Dibenzyl-sulphide: Radioactive studies. Radioactive isotope procedures for special types of chemical problems. Detmng. the three individual components of mixtures of ——, dibenzyl-sulphoxide and dibenzyl-sulphone. Henriques and Margnetti, 80.

Dibenzyl-sulphone: Ditto, ditto. Henriques and Margnetti, 80.

Dibenzyl-sulphoxide: Ditto, ditto. Henriques and Margnetti, 80.

1 : 1-Dichloro-2 : 2-bis(chlorophenyl)ethane: *See* DDD.

***Dichromate:** Detmng. sulphate in sodium ——. Wylie, 250.

Dictionary: Thorpe's —— of Applied Chemistry. 4th Edn. Vol. VIII. Meth-Oils, Essential. (Review), 499.

Diethyldithiocarbamate: Detmng. traces of copper in vegetable material with ——. Reith, Rameau, Mulder, Seekles, Kniphorst and Deijs, 555.

Differential manometer: Application of —— to measurement of fluid density and specific gravity with special reference to temperature effects. Cowherd, 80.

Digitalis: Effect of the anaesthetic and rate of injection of —— upon its lethal dose in cats. Holck, Kimura and Bartels, 449.

Digitoxin: Colorimetric assay of —— by modified Raymond method. Anderson and Chen, 362.

2 : 2′-Dihydroxy-5 : 5′-dichlorodiphenylmethane: Detmng. chlorine in ——. Jenkins, Waters and Beal, 31.

2 : 4-Dinitrophenylhydrazones: X-ray identification and crystallography of aldehydes and ketones as ——. Clark, Kaye and Parks, 407.

2 : 4-Dinitrophenylsemicarbazide. Reagent for aldehydes and ketones. McVeigh and Rose, 212.

Diolefines: Detmng. conjugated —— with chloromaleic anhydride. Putnam, Moss and Hall, 164.

Dipenta-erythritol: Detmng. —— in mixtures of penta-erythritol and ——. Wyler, 365.

Disodium-1 : 2-dihydroxy-benzene-3 : 5-disulphonate: Colorimetric detmn. of titanium with ——. Yoe and Armstrong, 552.

***Distillation:** Analysing hydrocarbon gases by low temperature ——. Hooper, 513.

Improved Kjeldahl trap and ammonia —— apparatus. Lovecy, 420.

***Dithizone:** Detmng. mercury with ——. Barnes, 469.

Detmng. traces of copper by extractive —— titration. Schonk, 492.

Dried egg powders: Detmng. free fatty acids in ——. Johnson and Kline, 67.

Drinks: Soft —— (Amendment) Order, 1947. 153; (Amendment No. 2) Order, 1947. 398; (Amendment No. 3) Order, 1947. 399.

Drugs: Detmng. sulphathiazole and other sulpha —— in honey. Shepard and Fisher, 448.

Dye: Accuracy of colorimetric instruments in —— strength detmns. Davies, Giles and Vickerstaff, 420.

Dysonian notation, 155.

E

Edible fats: Detmng. peroxide values of —— and oils. Iodimetric method. Lea, 255.

Edible Oils and —— (Control of Sales) (Amendment No. 2) Order, 1947. 208.

Edible Oils: Ditto, ditto, 208.

Detmng. peroxide values of edible fats and ——. Iodimetric method. Lea, 255.

Egg: Detmng. —— yolk in —— white. Cook and Mehlenbacher, 158.

powders: Detmng. free fatty acids in dried ——. Johnson and Kline, 67.

products: Estmng. chicken manure in —— from uric acid content. Duggan, 400.

Elaeostearic acid: Low-temperature crystallisation in detmng. component acids of liquid fats. III. Fats which contain —— as well as linoleic and oleic acids. Hilditch and Riley, 402.

***Electric heating:** Simple method for ——. Howes, 110.

Electrode: Amperometric titration of cyanide with silver nitrate, using rotating platinum ——. Laitinen, Jennings and Parks, 319.

cell: Improved glass —— assembly. Coates, 418.

*Rotating platinum ——. Morris, 298.

Electrolytes: Sensitivity of precipitation reactions in dilute ——. McAlpine, 116.

Electrometric analysis: three papers on ——. 90.

***Electrometric methods:** Some applications of —— in analysis. Carter, 94.

Electron diffraction and electron microscopic study of oxide films formed on metals and alloys at moderate temperatures. Phelps, Gulbransen and Hickman, 269.

Oxide films formed on alloys at moderate temperatures. —— and electron microscope study. Gulbransen, Phelps and Hickman, 223.

Electron microscope: Electron diffraction and —— study of oxide films formed on metals and alloys at moderate temperatures. Phelps, Gulbransen and Hickman, 269.

Oxide films formed on alloys at moderate temperatures. Electron diffraction and —— study. Gulbransen, Phelps and Hickman, 223.

Electron microscopy: Comparison of optical and —— of pigments. Tilleard and Smith, 223.

Prepng. powdered materials for ——. Schuster and Fullam, 224.

Electroscope: Radioactive studies. Analytical procedure for measurement of long-lived sulphur ^{35}S, with Lauritzen ——, and comparison of —— with special Geiger counter. Henriques, Kistiakowsky, Margnetti and Schneider, 80.

***Electro-titrations:** Recent developments in apparatus for *p*H measurements and ——. Lauchlan, 90.

Elektron: Potentiometric detmn. of aluminium in magnesium alloys of —— type. Poljak, 320.

Enumeration System: New Notation and —— for Organic Compounds. Dyson. (Review), 176.

Error(s) in spectrochemical (flame) analysis. Oertel and Stace, 497.

Source of —— in Gutzeit method for arsenic. Goldstone, 159.

Essential oils: Chemical microscopy of ——. IV. Cassia and cinnamon oils. Greene, 489.

Ethanolamine: Micro-detmn. of serine and —— in phospholipid hydrolysates. Burmaster, 23.

Ether(s): Analysing boron trifluoride in organic liquids (——). Walters and Miller, 32.

*Estmng. volatile matter content of propellant explosives. 2. Estmng. ethyl alcohol and ——. Bonner, 47.

Ethyl alcohol: Detmng. low concentrations of —— in aqueous solutions. Tschalov and Volskaja, 313.

Estmng. —— in blood. Rochat, 450.

*Estmng. volatile matter content of propellant explosives. 2. Estmng. —— and ether. Bonner, 47.

*Stability of cobaltous thiocyanate complex in —— - water mixtures and photometric detmn. of cobalt. Uri, 478.

***Evaporation** from beakers with radiant heater. Howes, 111.

Experimentation: Industrial. Brownlee. 2nd Edn. (Review), 422.

Explosion in detmng. cobalt as potassium coaltinitrite. Broughton, Laing and Wentworth, 410.

***Explosives:** Estmng. volatile matter content of propellant ——. 3. Estmng. acetone. Bonner, 434.

*Ditto, ditto. 2. Estmng. ethyl alcohol and ether. Bonner, 47.

Extinction corrections: Detmng. —— in infra-red analysis of gaseous hydrocarbon mixtures. Lee, 224.

F

Fat(s): Chemical Constitution of Natural ——. Hilditch. 2nd Edn. (Review), 500.

detmn. in dairy products. Janse, 485.

Detmng. peroxide values of edible —— and oils. Iodimetric method. Lea, 255.

Edible Oils and —— (Control of Sales) (Amendment No. 2) Order, 1947. 208.

Low-temperature crystallisation in detmng. component acids of liquid ——. I. —— in which oleic and linoleic acids are major components. Hilditch and Riley. II. —— which contain linolenic as well as linoleic and oleic acids. Gunstone and Hilditch. III. —— which contain elaeostearic as well as linoleic and oleic acids. Hilditch and Riley, 402.

South African fish products. XXII. Periodic acid as reagent in analysing unsaponifiable fraction of natural ——. Karnovsky and Rapson, 22.

Fat(s)—*continued*
*Spectrophotometric detmn. of small proportions of linolenic acid in ——. Hilditch and Shrivastava, 527.
Fatty acids: Detmng. free —— in dried egg powders. Johnson and Kline, 67.
Isolating and identifying —— as bis-(*p*-dimethyl-aminophenyl)-ureides. Breusch and Ulusoy, 364.
of corn (maize) oil. Baur, jun., and Brown, 448.
their Chemistry and Physical Properties. Markley. (Review), 557.
Fatty materials: Colorimetric detmn. of DDT in milk and ——. Schechter, Pogorelskin and Haller, 360.
Fatty oils: Puerto Rican ——. VI. Characteristics and composition of "Molinillo" seed oil. Asenjo, Goyco and Martinez-Pico, 485.
***Feed waters:** Photometric detmn. of dissolved oxygen in condensates and —— by means of starch-iodide complex. Bairstow, Francis and Wyatt, 340.
Feeding Stuffs (Regulation of Manufacture) (Amendment No. 10) Order, 1946. 64.
Ferrocyanide: Volumetric detmn. of mixtures of —— and arsenious acid. Lang, 551.
Ferro-molybdenum: Phenylanthranilic acid in volumetric detmn. of molybdenum in steel and ——. Fogelson and Kalmikova, 413.
Ferro-tungsten: Bivalent chromium solution for volumetric detmn. of tungsten. Detmng. tungsten in tungsten concentrates and ——. Tschernichov and Gorjuschina, 371
Ferrous ethylenediamine sulphate as oxidimetric standard. Caraway and Oesper, 491.
***Filter thimble:** Micro ——. Bowden, 542.
Fish products: South African ——. XXII. Periodic acid as reagent in analysing unsaponifiable fraction of natural fats. Karnovsky and Rapson, 22.
Flame analysis : Errors in spectrochemical analysis (——). Oertel and Stace, 497.
method of spectrochemical analysis. Roach, 174.
Flour Confectionery (Control and Max. Prices) (Amendment) Order, 1947. 207.
*Rapid qualitative test for added iron in ——. Pringle, 393.
Flue gas: Automatic detmn. of sulphur trioxide in ——. Gillham, 322.
Detmng. oxides of sulphur in ——. Gillham, 321.
***Fluorescence quenching** in solutions. Bowen, 379.
Fluoride: Colorimetric detmn. of ——. Monnier, Rusconi and Wenger, 117.
***Fluorimeter** design. Bowen, 377.
***Fluorimetric analysis:** Applications of —— to study of pterins. Simpson, 382.
Four papers, 377.
***Fluorimetry:** Applications of —— in vitamin analysis. Kodicek, 385.
Fluorine in Food Order, 1947. 398.
*Titrimetric estmn. of ——. Milton, Liddell and Chivers, 43.
Folin-Wu blood filtrates : Detmng. sulphonamides in tungstic acid blood filtrates (——). Schmidt, 25.
Food(s): Chemical Composition of ——. McCance and Widdowson. 2nd Edn. (Review), 81.
*Chemical estmn. of nicotinic acid in cereals and other ——. James, Norris and Wokes, 327.
Colorimetric detmn. of iron and manganese in ——. Kniphorst, 446.
Colorimetric estmn. of methionine in proteins and ——. Horn, Jones and Blum, 210.

Food(s)—*continued*
*Detmng. copper in ——. High, 60.
Fluorine in —— Order, 1947. 398.
Labelling of —— Order, 1946. 65.
Labelling of —— (Amendment) Order, 1947. 208; (Amendment No. 2) Order, 1947. 543.
Labelling of —— (General Licence) Order, 1947. 398.
Microbiological estmn. of methionine in proteins and ——. Horn, Jones and Blum, 211.
Polarographic detmn. of tin in —— and biological materials. Godar and Alexander, 111.
Standards (Mustard) (No. 2) (Amendment) Order, 1947. 208.
The Nation's ——. Survey of Scientific Data. Bacharach and Rendle. (Review), 271.
***Foodstuffs:** Chromatographic adsorption method for estmng. provitamin-A content of ——. Ramasarma, Hakim and Rao, 194.
Hydrolysis procedures for estmng. tryptophan in proteins and —— by microbiological procedure. Greenhut, Schweigert and Elvehjem, 25.
Microbiological estmn. of methionine in proteins and ——. Riesen, Schweigert and Elvehjem, 69.
Microbiological estmn. of methionine in proteins and ——. Horn, Jones and Blum, 211.
*Polarographic detmn. of lead in ——. Jones and Brasher, 423.
Semi-micro detmn. of calcium in ——. Kniphorst, 159.
Forensic Chemistry. Rhodes. 2nd Edn. (Review), 127.
Formic acid: Colorimetric micro-detmn. of ——. Grant, 490.
Fractionation: New principle of —— of rare earths with nitrilo-triacetate. Separating lanthanum and cerium. Beck, 74.
Friedrich method: Micro-Kjeldahl detmn. of nitrogen in gramicidin and tryptophan. Comparison of Gunning-Arnold-Dyer and ——s. White and Secor, 263.
Fructose: Identifying ——. Neuberg and Mandl, 263.
Fruit(s): Canned —— and Vegetables (Amendment No. 2) Order, 1947. 543.
Detmng. and isolating organic acids in ——. Isherwood, 261.
Detmng. DDT deposits on ——, vegetables and vegetation. Carter and Hubanks, 401.
***Furan:** Analytical methods applicable to —— and its derivatives. Angell, 178.
Furfural: Analysing —— -water solutions. Griswald, Klecka and West, 165.
***Fusion:** Factors affecting sodium carbonate —— of beryl. Osborn, 475.

G

Gallium: Detmng. —— in silicate rocks. Sandell, 457.
***Gas** analysis : Apparatus for —— by soap film method. Gooderham, 520.
*analysis : Procedure for —— at low pressures. Ransley, 504.
mixtures : Identifying sulphur compounds in ——. Oldach and Field, 219.
permeability : Isostatic method for detmng. —— of sheet materials. Davis, 81.
Gasoline: Detmng. tetra-ethyl lead in —— by X-ray absorption. Sullivan and Friedman, 269.

Gasoline—*continued*
Micro-detmn. of tetra-ethyl lead in ——. Gordon and Burdett, 454.
Picric acid method of detmng. aromatic content of aviation ——. Gambrill and Martin, 73.

Geiger counter: Radioactive studies. Analytical procedure for measurement of long-lived sulphur ^{35}S, with Lauritzen electroscope, and comparison of electroscope with special ——. Henriques, Kistiakowsky, Margnetti and Schneider, 80.

Gelatin: Edible —— (Control) Order, 1947. 66. (Amendment) Order, 1947. 309.

***Glass:** Analysing nickel - cobalt - iron alloys used in —— -to-metal seals. Chirnside, Cluley and Proffitt, 351.
Detmng. boric oxide in ——. Hollander and Rieman, 317.
*Detmng. lead as iodate in —— analysis. Proffitt and Chirnside, 205.

Glass electrode cell: Improved —— assembly. Coates, 418.

Glucose: Estmng. penicillin in serum. Use of ——, phenol red and serum water. Fleming and Smith, 548.
Estmng. pentose in presence of large quantities of ——. Brown, 164.
Iodimetric estmn. of —— in urine and blood. Noyons, 210.

Glycerol dichlorohydrin: Activated ——. Colorimetric reagent for vitamin A. Sobel and Werbin, 27.

Glycogen: Colorimetric estmn. of ——. Morris, 164.
Estmng. —— in whole blood and white blood cells. Wagner, 162.

Glycols: Solid polyethylene —— (Carbowax compounds). Quantitative detmn. in biological materials. Shaffer and Critchfield, 404.

Gold: Detmng. —— by back-titration. Tzobar, 373.
Detmng. —— with ascorbic acid. Stathis and Gatos, 123.

Gossypol: Effect of acetic acid on spectrophotometric estmn. of —— in aqueous alcoholic solution. Smith, 32.

Gramicidin: Micro-Kjeldahl detmn. of nitrogen in —— and tryptophan. Comparison of Gunning-Arnold-Dyer and Friedrich methods. White and Secor, 263.

***Grass:** Detmng. carotene in dried ——. Nelson, 200.

***Growth factors:** Computation of microbiological assays of amino acids and other ——. Wood, 84.

***Gums:** Separation of crystals and —— on micro or semimicro scale. Bacharach, 244.

Gunning-Arnold-Dyer method: Micro-Kjeldahl detmn. of nitrogen in gramicidin and tryptophan. Comparison of —— and Friedrich method. White and Secor, 263.

Gutzeit method: Source of error in —— for arsenic. Goldstone, 159.

H

Haemoglobin: Detmng. carbon monoxide in blood and total and active —— by carbon monoxide capacity. van Slyke, Hiller, Weisiger and Cruz, 257.
in meat scraps and tankage. Reiser, 446.

Hafnium: Fractional separation of —— and zirconium by means of triethyl phosphate. Willard and Freund, 216.

Halide ions: Semimicro detcn. of thiocyanate and ——. Porter, 76.

Hardness: Colorimetric detmn. of —— of water. Dratschev, 460.
Detmng. total —— of water by means of soap solution. Teterkin, 375.
New simple titration methods for detmng. —— of water. Schwarzenbach, Biedermann and Bangerter, 322.

***Heating:** Simple method for electric ——. Howes, 110.

***Hexachlorocyclohexane:** Detmng. small amounts of —— (benzene hexachloride). Howard, 427.

Hexuronic acids: New specific colour reaction of ——. Dische, 365.

High-frequency oscillators: Use of —— in titrations and analyses. Jensen and Parrack, 78.

Histidine: Step-photometric detmn. of ——. Schmid, 486.

Honey: Detmng. sulphathiazole and other sulpha drugs in ——. Shepard and Fisher, 448.

Hormones: Polarographic estmn. of steroid ——. Polarography of neutral 17-ketosteroids in urinary extracts. Barnett, Henly and Morris, 26.

***Human body:** Distribution of barbiturates in the ——. Andrew and Neubauer, 21.

***Hydrocarbon(s):** Analysing —— gases by low temperature distillation. Hooper, 513.
Characterisation of pure ——. Refractometric analysis as key to evaluation of structure. Wendland, 114.
mixtures: Detmng. extinction corrections in infra-red analysis of ——. Lee, 224.
mixtures: Quantitative analysis of —— by means of Raman spectra. Rosenbaum, Martin and Lauer, 261.
Phase-volumetric analysis of mixtures of benzine and aromatic ——. de Goeij, 549.

Hydrogen: Detmng. "active" ——. Braude and Stern, 213.
Detmng. —— in liquid steel. Wells and Barraclough, 457.
Traces of oxygen in —— or nitrogen. Liebhafsky and Winslow, 267.

Hydrogen peroxide-treated peptone in media for microbiological detmn. of amino acids. Lyman, Moseley, Wood and Hale, 70.

Hydrogen sulphide: Conversion of organic sulphur to —— for analysis. Field and Oldach, 115.
Detmng. —— in gases. Field and Oldach, 172.
Simultaneous detmn. of —— and carbon dioxide in continuous gas stream. Blohm and Riesenfeld, 495.

Hydrogenation: Qualitative elementary analysis [of organic substances] by ——. Slooff, 163.

Hydrolysis procedures for estmng. tryptophan in proteins and foodstuffs by microbiological procedure. Greenhut, Schweigert and Elvehjem, 25.

Hydroperoxides: Detmng. —— in rubber and synthetic polymers. Laitinen and Nelson, 369.

Hydroxides: Oxidation of metallic —— by current of air. Fireman, 214.

8-Hydroxyquinoline: Detmng. uranium with ——. Claassen and Visser, 123.
Quantitative detmn. of tungsten, molybdenum, and vanadium, alone and in presence of ferric iron, with ——. Niericker and Treadwell, 214.

Hypochlorous acid: Comparative estmns. of carbonyl and carboxyl groups in chromium trioxide and —— oxycelluloses and oxyxylans. Meesook and Purves, 72.

Ice cream: Circular 69/47. Ministry of Health, 154.

  (Heat Treatment, etc.) Regulations, 1947. 153.

Identification of crystalline organic compounds by X-ray analysis. de Lange and Houtman, 406.

  X-ray —— and crystallography of aldehydes and ketones as 2 : 4-dinitrophenylhydrazones. Clark, Kaye and Parks, 407.

Impurities: Limit tests for ——. Reimers and Gottlieb, 485.

  Limit Tests for ——. Reimers and Gottlieb. (Review), 500.

Index to the Literature on Spectrochemical Analysis, Part II, 1940 to 1945. Scribner and Meggers. (Review), 325.

***Indicator** changing at *p*H 0·5 for control of sulphide pptn. Andrew, 481.

  properties of derivatives of 4′-nitrophenylazo-1-naphthol. Ferber, 72.

Indole in shrimp. Duggan and Strasburger, 545.

Infra-red analysis: Detmng. extinction corrections in —— of gaseous hydrocarbon mixtures. Lee, 224.

Inorganic analysis: Method of semi-quantitative ——. Wenger, Monnier and Piguet, 116.

Institute of Petroleum: Standard Methods of Testing Petroleum and its Products. 8th Edn. 254.

Instruments: Accuracy of colorimetric —— in dye strength detmns. Davies, Giles and Vickerstaff, 420.

Iodides: Identifying aryl ——. Nichol and Sandin, 212.

Ion exchange resins in study of chrome liquors. Adams, 169.

***Iron:** Analysing nickel - cobalt - —— alloys used in glass-to-metal seals. Chirnside, Cluley and Proffitt, 351.

  Cacotheline in volumetric analysis. II. Detmng. tervalent —— by titration with stannous chloride solution. Kutschment and Gengrinovitsch, 492.

  Colorimetric detmn. of —— and manganese in foods. Kniphorst, 446.

  Colorimetric detmn. of —— in brass and bronze. Goodman, 494.

  Detmng. copper in cast —— and steels with quinaldic acid. Flagg and Vanas, 121.

  Detmng. —— in presence of cobalt. Brown, 122.

  Detmng. nickel and cobalt in —— ores with preliminary removal of —— by means of sodium fluoride. Tananaev and Silnitschenko, 373.

  Detmng. oxygen in —— and steel. Vigna, 456.

  Detmng. traces of —— in bacteriological media. Tasman and Smith, 555.

  *Direct colorimetric detmn. of tungsten in cast ——. Westwood and Mayer, 464.

  Entrainment of cobalt and sulphur in —— separations. Pinkney, Dick and Young, 122.

  Polarographic investigations of oxalate, citrate, and tartrate complexes of ferric and ferrous ——. Lingane, 370.

  Pyrophosphoric acid in detmng. nickel in cast —— and steel. Kononiuk, 413.

  Quantitative detmn. of tungsten, molybdenum, and vanadium, alone and in presence of ferric ——, with 8-hydroxyquinoline. Niericker and Treadwell, 214.

  *Rapid qualitative test for added —— in flour. Pringle, 393.

Iron oxide: Detmng. rates of de-oxidation of materials in reducing gases. Barrett and Wood, 318.

***Iron pyrites:** Detmng. copper and arsenic in —— by internal electrolysis. Davies and Key, 17.

Isopropyl ether: Extracting vanadium from aqueous solutions by ——. Lingane and Meites, jun., 216.

Isostatic method for detmng. gas permeability of sheet materials. Davis, 81.

Isotope dilution procedures: Radioactive studies. Radioactive —— for special types of chemical problems. Detmng. the three individual components of mixtures of dibenzyl-sulphide, -sulphoxide and -sulphone. Henriques and Margnetti, 80.

J

Jams: Detmng. specific gravity of extracts of ——, syrups, etc., based on density gradient. van der Kamer and Jansen, 399.

Johnson, Matthey and Co., Ltd.: Standardised Substances for Spectrography, Chemical Analysis and Research, 254.

K

Ketones: 2 : 4-Dinitrophenylsemicarbazide. Reagent for aldehydes and ——. McVeigh and Rose, 212.

  X-ray identification and crystallography of aldehydes and —— as 2 : 4-dinitrophenylhydrazones. Clark, Kaye and Parks, 407.

17-Ketosteroids: Polarographic estmn. of steroid hormones. Polarography of neutral —— in urinary extracts. Barnett, Henly and Morris, 26.

Kjeldahl detmn. of nitrogen. Elimination of distillation. Marcali and Rieman, 163.

Kjeldahl trap: Improved —— and ammonia distillation apparatus. Lovecy, 420.

L

Labelling of Food Order, 1946. 65.

  of Food (Amendment) Order, 1947. 208; (Amendment No. 2) Order, 1947. 543.

  of Food (General Licence) Order, 1947. 398.

Laboratory animal: Maintenance of —— colonies. New York Academy of Sciences, 501.

Lanthanum: New principle of fractionation of rare earths with nitrilo-triacetate. Separating —— and cerium. Beck, 74.

Lauritzen electroscope: Radioactive studies. Analytical procedure for measurement of long-lived sulphur ^{35}S, with ——, and comparison of —— with special Geiger counter. Henriques, Kistiakowsky, Margnetti and Schneider, 80.

Lead: Applying polarographic analysis to production control in non-ferrous metallurgy. I. Detmng. copper, —— and zinc impurities in metallic cadmium. Pletenev, Arefyeva, Tal and Dubovitzkaja, 415.

  Ditto. III. Detmng. copper, bismuth, ——, cadmium and zinc impurities in metallic tin and —— - tin solders. Pletenev, Arefyeva, Tal and Dubovitzkaja, 416.

  Ditto. IV. Detmng. impurities in metallic ——. Pletenev, Arefyeva, Tal and Dubovitzkaja, 416.

Lead—*continued*
  Ditto. V. Rapid detmn. of ——, cadmium and zinc in ores and refuse from ore-enrichment (zinc, —— and cadmium ores). Pletenev, Arĕfyeva, Tal and Dubovitzkaja, 416.
  *Detmng. —— as iodate in glass analysis. Proffitt and Chirnside, 205.
  Internal standard method of spectrographic analysis as applied to detmng. —— in high-purity zinc. Griffith and Kirkbride, 223.
  *Polarographic detmn. of —— in foodstuffs. Jones and Brasher, 423.
  Polarographic detmn. of —— in —— slags. Fainberg and Tal, 268.
  *Polarographic detmn. of tin, —— and zinc in phenol. Wilson and Hutchinson, 149.
***Lead-base alloys:** Spot-tests for detcng. alloying elements in ——. Evans and Higgs, 105.
Lead compounds: Volumetric detmn. of organic ——. Hein, Klein and Mesée, 549.
Leaf ash: Rapid semi-micro volumetric detmn. of potash in ——. Chapman, 374.
Liebermann-Burchard reaction: Photometric detmn. of total cholesterol in plasma or serum by modified ——. Saifer and Kammerer, 27.
Limit tests for impurities. Reimers and Gottlieb, 485.
  for Impurities. Reimers and Gottlieb. (Review), 500.
Linoleic acid: Detmng. —— in cholesteryl linoleate. Front and Daubert, 314.
  Low-temperature crystallisation in detmng. component acids of liquid fats. I. Fats in which oleic and ——s are major components. Hilditch and Riley. II. Fats which contain linolenic as well as —— and oleic acid. Gunstone and Hilditch. III. Fats which contain elæostearic as well as —— and oleic acids. Hilditch and Riley, 402.
Linolenic acid: Ditto, ditto. Gunstone and Hilditch, 402.
  *Spectrophotometric detmn. of small proportions of —— in fats. Hilditch and Shrivastava, 527.
Literature: Index to the —— on Spectrochemical Analysis, Part II, 1940 to 1945. Scribner and Meggers. (Review), 325.

M

Magnesia: Detmng. calcium in magnesite and fused ——. Hazel and Eglof, 168.
  *Detmng. calcium oxide in low-lime refractory ——. Gilbert, 482.
Magnesite: Detmng. calcium in —— and fused magnesia. Hazel and Eglof, 168.
Magnesium: Separating calcium from —— by oxalate method in samples of high —— -calcium ratio. Wright and Delaune, 118.
Magnesium alloys: Potentiometric detmn. of aluminium in —— of Elektron type. Poljak, 320.
Magnesium ammonium phosphate hexahydrate: Solubility of ——. Uncles and Smith, 118.
Maize: Fatty acids of corn (——) oil. Baur, jun., and Brown, 448.
Manganese: Colorimetric detmn. of iron and —— in foods. Kniphorst, 446.
  *Detmng. small quantities of —— in caustic soda. Ethrington and Hughes, 472.
Manometer: Application of differential —— to measurement of fluid density and specific gravity with special reference to temperature effects. Cowherd, 80.

Manometer—*continued*
  Molecular weight detmn. with micro- ——. Young and Taylor, 497.
  Vacuum micro- ——. Young and Taylor, 497.
Manure: Estmng. chicken —— in egg products from uric acid content. Duggan, 400.
Meat: Haemoglobin in —— scraps and tankage. Reiser, 446.
  Products, Canned Soup and Canned —— (Control and Max. Prices) (Amendment No. 3) Order, 1946. 64.
  Products and Canned Meat (Control and Max. Prices) Order, 1947. 111; (Amendment) Order, 1947. 153.
Media: Hydrogen-peroxide-treated peptone in —— for microbiological detmn. of amino acids. Lyman, Moseley, Wood and Hale, 70.
Melamine: Identifying ——. Candlin, 491.
  resin: Analysis of dilute —— solutions. Ivarsson and Steenberg, 32.
Mercurous iodate: Micro-detmn. of chlorides by means of ——. Avaliani, 370.
Mercury: Analysing mixtures of mercurous and mercuric —— and sulphuric acid. Warshowsky and Elving, 493.
  *Detmng. —— with dithizone. Barnes, 469.
  *Estmng. —— compounds in the atmosphere. Milton and Duffield, 11.
  *Estmng. traces of —— in urine. Milton and Hoskins, 6.
  Polarography of —— solutions. Poma, 552.
  reductor: Behaviour of uranyl solutions in ——. Caley and Rogers, 216.
***Metal(s):** Analysing nickel-cobalt-iron alloys used in glass-to- —— seals. Chirnside, Cluley and Proffitt, 351.
  Electron diffraction and electron microscopic study of oxide films formed on —— and alloys at moderate temperatures. Phelps, Gulbransen and Hickman, 269.
  *System of qualitative analysis for common —— in presence of phosphates, using ammonium benzoate. Austin, 443.
Metallurgy: Applying polarographic analysis to production control in non-ferrous ——. Pletenev, Arefyeva, Tal and Dubovitzkaja, 415.
Methionine: Colorimetric estmn. of —— in proteins and foods. Horn, Jones and Blum, 210.
  Microbiological estmn. of amino acids. III. ——. Lyman, Moseley, Butler, Wood and Hale, 209.
  Microbiological estmn. of —— in proteins and foods. Horn, Jones and Blum, 211.
  Microbiological estmn. of —— in proteins and foodstuffs. Riesen, Schweigert and Elvehjem, 69.
N^1-Methylnicotinamide: Detmng. —— in urine. Huff and Perlzweig, 363.
***Microbiological assays:** Computation of —— of amino acids and other growth factors. Wood, 84.
Micromanometer: Molecular weight detmn. with vacuum ——. Young and Taylor, 497.
  Vacuum ——. Young and Taylor, 497.
Microscope, Theory and Applications. Wredden. (Review), 557.
Microscopical differentiation of common mustard seeds with polarised light. Keenan, 448.
Microscopy: Comparison of optical and electron —— of pigments. Tilleard and Smith, 223.
Mikroanalyse: Quantitative organische ——. Pregl. 5th Edn. (Review), 461.
Milk: Colorimetric detmn. of DDT in —— and fatty materials. Schechter, Pogorelskin and Haller, 360.

Milk—*continued*
Detmng. DDT in —— by detmng. organic chlorine. Carter, 361.
Modified Scharer phosphatase test for —— and soft cheeses. Horwitz, 544.
*powder: Chapman and McFarlane method for estmng. reducing groups in ——. Lea, 336.
Minerals: Identifying and estmng. montmorillonite group of ——, with special reference to soil clays. MacEwan, 218.
Ministry of Food Orders, etc.:
Bread, 398.
Fats, edible, 208.
Feeding stuffs, 64.
Flour confectionery, 207.
Fluorine in food, 398.
Food standards, 208.
Fruit, canned, 543.
Gelatin, 66, 309.
Ice cream, 153.
Labelling of food, 65, 208, 398, 543.
Meat, canned, 64, 111, 153.
Meat products, 64, 111, 153.
Mustard, 208.
Oils, edible, 208.
Pickles, 309.
Preserves, 398.
Sauces, 309.
Soft drinks, 153, 398, 399.
Soup, canned, 64.
Vegetables, canned, 543.
Ministry of Health: Circular 69/47. Ice Cream, 154.
Molecular still: Simple laboratory falling-film ——. Farmer and Sutton, 174.
Molecular weight detmn. with vacuum micromanometer. Young and Taylor, 497.
Molybdenum: Colorimetric detmn. of —— as thiocyanate complex. Tananaev and Lochvitzkaja, 455.
*Detmng. tungsten in steel, using toluene-3 : 4-dithiol. Removal of —— interference by selective extraction. Bagshawe and Truman, 189.
*Microchemical detmn. of —— in steel. Wells and Pemberton, 185.
Phenylanthranilic acid in volumetric detmn. of —— in steel and ferro- ——. Fogelson and Kalmikova, 413.
Quantitative detmn. of tungsten, ——, and vanadium, alone and in presence of ferric iron, with 8-hydroxyquinoline. Niericker and Treadwell, 214.
Molybdenum blue: Constitution of ——. Treadwell and Schaeppi, 117.
Molybdi-vanadate method: Detmng. serum phosphate by ——. Simonsen, Wertman, Westover and Mehl, 312.
Monochloroacetic acid: New method of detcng. and detmng. ——. Ramsey and Patterson, 405.
Montmorillonite: Identifying and estmng. —— group of minerals, with special reference to soil clays. MacEwan, 218.
Morgan method of soil testing. IV. Use of Spekker absorptiometer for estmng. phosphate. Tinsley and Pizer, 124.
Muscle: Tocopherol content of skeletal ——; comparison of chemical and bio-assay methods. Kaunitz and Beaver, 161.
Mustard: Food Standards (——) (No. 2) (Amendment) Order, 1947, 208.
Microscopical differentiation of common —— seeds with polarised light. Keenan, 448.
Mustard gas: Iodimetric micro-titration for ——. Kinsey and Grant, 315.

N

Narceine: Detmng. —— in —— sodium salicylate with Reinecke's salt. van Arkel and Kramer, 547.
New York Academy of Sciences: Maintenance of laboratory animal colonies, 501.
***Nickel:** Analysing —— - cobalt - iron alloys used in glass-to-metal seals. Chirnside, Cluley and Proffitt, 351.
Detmng. —— and cobalt in iron ores with preliminary removal of iron by means of sodium fluoride. Tananaev and Silnitschenko, 373.
Detmng. —— in cobalt steels and alloys. Kirtchik, 553.
Detmng. phosphorus in austenitic chromium - —— steels. Methods of analysis of Metallurgy Division of B.I.S.R.A., 412.
*Pptng. aluminium and separating it from cobalt, chromium, ——, or zinc by means of ammonium benzoate. Smales, 14.
Pyrophosphoric acid in detmng. —— in cast iron and steel. Kononiuk, 413.
*Titration of minute amounts of ——. Evans, 110.
Nickelous complexes: Metallic complexes of tartrates and citrates; structure and behaviour in dilute solutions I. Cupric and ——. Bobtelsky and Jordan, 213.
Nicotine: Titrimetric detmn. of di-acidic bases (——, quinine) in mixtures with mono-acidic bases, and simplified assay for ——. Trautner and Neufeld, 160.
Nicotinic acid: Chemical estmn. of ——. Mueller and Fox, 362.
*Chemical estmn. of —— in cereals and other foods. James, Norris and Wokes, 327.
Microbiological estmn. of —— using *Proteus* HX19. Grossowicz and Sherstinsky, 362.
Purity of —— used in assays. 501.
Yeast microbiological method for estmng. ——. Williams, 312.
Niobium: Separating —— and tantalum from titanium. Oschman, 373.
***Nitrate(s):** Detmng. —— in water. Allerton, 349.
fusion: Rare-earth metal oxides. II. Study of oxide pptn. by ——. Marsh, 169.
Polarographic analysis of nitrite and nitrite - —— mixtures. Keilin and Otvos, 268.
Polarographic analysis of nitrite and nitrite - —— mixtures. Keilin and Otvos, 268.
Nitration in sulphuric acid. III. Influence of nitric acid and other substances on oleum - water titration. Brand, 169.
Nitric acid: Nitration in sulphuric acid. III. Influence of —— and other substances on oleum-water titration. Brand, 169.
Nitrilo-triacetate: New principle of fractionation of rare earths with ——. Separating lanthanum and cerium. Beck, 74.
Nitrite: Polarographic analysis of —— and —— - nitrate mixtures. Keilin and Otvos, 268.
Nitro-Captax: 6 - Nitro - 2-mercaptobenzothiazole (——) as reagent for identifying alkyl halides. Cutter and Golden, 550.
Nitrogen: Kjeldahl detmn. of ——. Elimination of distillation. Marcali and Rieman, 163.
Micro-Kjeldahl detmn. of —— in gramicidin and tryptophan. Comparison of Gunning-Arnold-Dyer and Friedrich methods. White and Secor, 263.
*Oxidation of —— during micro-combustion of organic substances. Heron, 142.

Nitrogen—*continued*
  Traces of oxygen in hydrogen or ——. Liebhafsky and Winslow, 267.
6-Nitro-2-mercaptobenzothiazole ("Nitro-Captax") as reagent for identifying alkyl halides. Cutter and Golden, 550.
***Nitromethane:** Detmng. —— in air. Wilson and Hutchinson, 432.
4′-Nitrophenylazo-1-naphthol: Indicator properties of ——. Ferber, 72.
Nitrosocresol: Estmng. cobalt in biological materials with ——. Ellis and Thompson, 113.
Non-ferrous metallurgy: Applying polarographic analysis to production control in ——. Pletenev, Arefyeva, Tal and Dubovitzkaja, 415.
Notation: Dysonian ——. 155.
  New —— and Enumeration System for Organic Compounds. Dyson. (Review), 176.
Novocaine: Microscopical identification of butacaine sulphate (Butyn) and procaine hydrochloride (——). Keenan, 547.
Nucleotides: Rapid fluorimetric estmn. of total pyridine —— in red blood cells. Levitas, Robinson, Rosen, Huff and Perlzweig, 363.

O

Obituaries:
  Battye, H. G., 131.
  Blackie, J. J., 35, 131.
  Boizot, G. E., 131.
  Bruce, A., 131, 132.
  Buchanan, J. L., 131, 132.
  Butterfield, W. J. A., 131, 132.
  Dracass, W. R., 131, 132.
  Edwards, G. W., 131, 132.
  Ellison, R., 229.
  Firth, H., 131, 132.
  Foster, J. A., 131, 132.
  Frankland, P. F., 131, 132.
  Garbutt, C. D., 131.
  Heywood, A. D., 131, 132.
  Hinks, E., 131, 132.
  Hopkins, Sir Frederick G., 229.
  Jones, P. H., 131, 132.
  Liverseege, J. F., 131, 132.
  McGrath, T., 131.
  Mason, F. A., 423.
  Morell, R. S., 35, 131, 133.
  Naidu, S. R., 177.
  Needs, F. E., 131, 133.
  Nierenstein, M., 131.
  Roberts, W. H., 36, 131, 133.
  Sherlock, A., 423.
  Smith, H. P., 423.
  Spilsbury, Sir Bernard H., 503.
  Vakil, K. H., 131.
  Warburton, G. H., 131.
  Watkinson, S., 131, 133.
Oestrone: Polarographic detmn. of —— in urine. Bjornson and Ottesen, 403.
Oil(s): Chemical microscopy of essential ——. IV. Cassia and cinnamon ——. Greene, 489.
  Detmng. peroxide values of edible fats and ——. Iodimetric method. Lea, 255.
  Edible —— and Fats (Control of Sales) (Amendment No. 2) Order, 1947. 208.
  Fatty acids of corn (maize) ——. Baur, jun., and Brown, 448.
  Puerto Rican fatty ——. VI. Characteristics and composition of "Molinillo" seed ——. Asenjo, Goyco and Martinez-Pico, 485.
  Rancidity of olive ——. Kaloyereas, 256.

Oil(s)—*continued*
  Seed —— of some Sudan Caesalpinioideae. Grindley, 111.
Olefines: Micro-detmn. of gaseous ——. Pyke, Kahn and LeRoy, 418.
Oleic acid: Low-temperature crystallisation in detmng. component acids of liquid fats. I. Fats in which —— and linoleic acid are major components. Hilditch and Riley. II. Fats which contain linolenic as well as linoleic acid and ——. Gunstone and Hilditch. III. Fats which contain elæostearic as well as linoleic and ——s. Hilditch and Riley, 402.
Oleum: Nitration in sulphuric acid. III. Influence of nitric acid and other substances on —— -water titration. Brand, 169.
Olive oils: Rancidity of ——. Kaloyereas, 256.
Ores: Applying polarographic analysis to production control in non-ferrous metallurgy. V. Rapid detmn. of lead, cadmium and zinc in —— and refuse from —— -enrichment (zinc, lead and cadmium ——). Pletenev, Arefyeva, Tal and Dubovitzkaja, 417.
  Detmng. nickel and cobalt in iron —— with preliminary removal of iron by means of sodium fluoride. Tananaev and Silnitschenko, 373.
Organic Chemistry. Karrer. 3rd English Edn. (Review), 421.
Organic compounds: Identifying crystalline —— by means of X-ray analysis. de Lange and Houtman, 406.
  Identifying —— by chromium target X-ray diffraction powder patterns. Matthews and Michell, 174.
Organic liquids: Analysing boron trifluoride in —— (ethers). Walters and Miller, 32.
Organic Preparations. Weygand. (Review), 33.
Organic reagents: Analytical reactions with ——. Steigmann, 28.
Organoselenium compounds: Analysing ——. McCullough, Campbell and Krilanovich, 30.
Organosilicon compounds: Detmng. silicon in relatively non-volatile ——. Gilman, Clark, Wiley and Diehl, 264.
Oscillators: Use of high-frequency —— in titrations and analyses. Jensen and Parrack, 78.
***Oscillograph:** Applying cathode ray —— to polarography: general lay-out and uses of the cathode ray polarograph. Airey, 304.
  *Applying cathode ray —— to polarography: underlying principles. Randles, 301.
Oxalate: Polarographic investigations of ——, citrate, and tartrate complexes of ferric and ferrous iron. Lingane, 370.
***Oxalic acid** extractant in vitamin C assay. Wokes, 63.
Oxidation of metallic hydroxides by current of air. Fireman, 214.
  *of nitrogen during micro-combustion of organic substances. Heron, 142.
  Spectrophotometric changes during —— of vitamin A oils. Halpern, 78.
Oxide(s) films: Electron diffraction and electron microscopic study of —— formed on metals and alloys at moderate temperatures. Phelps, Gulbransen and Hickman, 269.
  films formed on alloys at moderate temperatures. Electron diffraction and electron microscope study. Gulbransen, Phelps and Hickman, 223.
  of sulphur: Detmng. —— in flue gas. Gillham, 321.
  Rare-earth metal ——. III. Pptn. from potassium hydroxide melts. Marsh 169.

Oxide(s)—*continued*
  Rare-earth metal ——. II. Study of —— pptn. by nitrate fusion. Marsh, 169.
Oxidimetric standard: Ferrous ethylenediamine sulphate as ——. Caraway and Oesper, 491.
Oxidising agents: Anomalous behaviour of some ——. McAlpine, 75.
Oximes: Chromatographic resolution of quinone ——. Gullstrom, Burchfield and Judy, 77.
Oxycelluloses: Comparative estmns. of carbonyl and carboxyl groups in chromium trioxide and hypochlorous acid —— and oxyxylans. Meesook and Purves, 72.
  Identifying acidic —— by means of aromatic amines and diamines. Krajčinović and Krajčinović, 366.
Oxygen: Analytical chemistry of rare earths. Active- —— detmn. Barthauer and Pearce, 217.
  Detmng. —— in iron and steel. Vigna, 456.
  Detmng. —— in presence of sulphite. Janssen, 496.
  *Photometric detmn. of dissolved —— in condensates and feed waters by means of starch-iodide complex. Bairstow, Francis and Wyatt, 340.
  Traces of —— in hydrogen or nitrogen. Liebhafsky and Winslow, 267.
Oxyxylans: Comparative estmns. of carbonyl and carboxyl groups in chromium trioxide and hypochlorous acid oxycelluloses and ——. Meesook and Purves, 72.

P

Paints: Volumetric detmn. of water in —— and varnishes. Swann, 170.
Paper: Detmng. available chlorine in ——. Birchard, 493.
  Disintegration of viscose in analysing —— fibres. Browning and Graff, 491.
Particle size by spectral transmission. Bailey, 222.
  Measuring distribution of —— in dispersed systems. Dotts, 324.
  Prepng. standard powders for reference in —— measurement. Gooden and Updike, jun., 270.
Penicillin(s): Chemical assay method for —— G. Sheehan, Mader and Cram, 313.
  Chemical assay of ——. Mundell, Fischbach and Eble, 488.
  *Chemistry of ——. Robinson, 274.
  Estmng. —— and other bacteriostatics. Fielding, 256.
  Estmng. —— in serum. Use of glucose, phenol red and serum water. Fleming and Smith, 548.
  Iodimetric assay of —— preparations. Alicino, 68.
  Its Properties, Uses and Preparations. Pharmaceutical Society of Great Britain. (Review), 225.
  *Micro-chromatographic detcn. and approximate detmn. of the different —— in a mixture. Goodall and Levi, 277.
  Three-hour physical development cup-plate assay for ——. Goyan, Dufrenoy, Strait, Pratt and Juntunen, 488.
Penta-erythritol: Detmng. dipenta-erythritol in mixtures of —— and dipenta-erythritol. Wyler, 365.
Pentose(s): Estmng. —— in presence of large quantities of glucose. Brown, 164.
  Micro-detmn. of —— by bisulphite method. Heines, 267.
Peptone: Hydrogen-peroxide-treated —— in media for microbiological detmn. of amino acids. Lyman, Moseley, Wood and Hale, 70.
Perchloric acid: Detmng. chromium by oxidation with ——. Schuldiner and Clardy, 410.
Percolator: Simple automatic ——. Lees, 496.
Periodic acid: South African fish products. XXII. —— as reagent in analysing unsaponifiable fraction of natural fats. Karnovsky and Rapson, 22.
Permeability: Isostatic method for detmng. gas —— of sheet materials. Davis, 81.
Peroxide values: Detmng. —— of edible fats and oils. Iodimetric method. Lea, 255.
Petrol: *See* Gasoline.
Petroleum: Standard Methods for Testing —— and its Products. Institute of Petroleum. 8th Edn., 254.
***pH:** Recent developments in apparatus for —— measurements and electro-titrations. Lauchlan, 90.
Pharmaceutical products: Assay for choline chloride in ——. Gakenheimer and Regnera, 23, 486.
Pharmaceutical Society of Great Britain: Penicillin : Its Properties, Uses and Preparations. (Review), 225.
Pharmacognosy: Textbook of ——. Wallis. (Review), 376.
Pharmacopoeia of the United States of America. 13th Revision (U.S.P. XIII). (Review), 498.
***Phenarsazine:** Detmng. micro-quantities of derivatives of ——. Barnes, 241.
***Phenol:** Identifying —— from synthetic and natural sources. Boorman, Daubney and Martin, 246.
  *Polarographic detmn. of tin, lead and zinc in ——. Wilson and Hutchinson, 149.
  Quantitative analysis of isomeric cresols and cresol- —— mixtures by U.V. absorption spectra of vapours. Robertson, Ginsburg and Matsen, 264.
Phenol red: Estmng. penicillin in serum. Use of glucose, —— and serum water. Fleming and Smith, 548.
Phenylanthranilic acid in volumetric detmn. of molybdenum in steel and ferro-molybdenum. Fogelson and Kalmikova, 413.
Phosphatase: Modified Scharer —— test for milk and soft cheeses. Horwitz, 544.
Phosphate(s): Analysing external deposits from boilers. II. Analysing deposits rich in ——. Edwards, 119.
  Detmng. serum —— by molybdi-vanadate method. Simonsen, Wertman, Westover and Mehl, 312.
  Morgan method of soil testing. IV. Use of Spekker absorptiometer for estmng. ——. Tinsley and Pizer, 124.
  *System of qualitative analysis for common metals in presence of ——, using ammonium benzoate. Austin, 443.
Phospholipid: Micro-detmn. of serine and ethanolamine in —— hydrolysates. Burmaster, 23.
***Phosphomolybdate:** Detmng. composition and constitution of ammonium —— and conditions affecting its pptn. Thistlethwaite, 531.
Phosphorus: Detmng. —— in austenitic chromium - nickel steels. Methods of analysis of the Metallurgy Division of B.I.S.R.A., 412.
Physical development: Three-hour —— cup-plate assay for penicillin. Goyan, Dufrenoy, Strait, Pratt and Juntunen, 488.
Pickles and Sauces Order, 1947. 309.

Picric acid method for detmng. aromatic content of aviation gasoline. Gambrill and Martin, 73.

Pigments: Comparison of optical and electron microscopy of ——. Tilleard and Smith, 223.

Plants: Detcng. alkaline earth metals in cell tissue of ——. Haller, 71.

Plasma: Photometric detmn. of total cholesterol in —— or serum by modified Liebermann-Burchard reaction. Saifer and Kammerer, 27.

Precision method for quantitative detmn. of calcium in blood ——. Salomon, Gabrio and Smith, 310.

Plating: Analysing brass ——. Roberts, 273.

baths : Polarographic analysis of brass —— and brass coatings. Viachirev, 268.

Platinum electrode: Amperometric titration of cyanide with silver nitrate, using rotating ——. Laitinen, Jennings and Parks, 319.

*Rotating ——. Morris, 298.

***Polarisation** end-points. Evans, 99.

Polarised light: Microscopical differentiation of common mustard seeds with ——. Keenan, 448.

***Polarograms:** Aids to accuracy in quantitative estmn. from simple ——. Lewis, 308.

***Polarograph(s):** Application of cathode ray oscillograph to ——y : general lay-out and uses of cathode ray ——. Airey, 304.

Operating characteristics of Sargent Model XX visible recording ——. Lingane, 496.

Remarks on ——. Davies, 273.

Polarographic analysis: Applying —— to production control in non-ferrous metallurgy. Pletenev, Arefyeva, Tal and Dubovitzkaja, 415.

***Polarographic determination** of tin, lead and zinc in phenol. Wilson and Hutchinson, 149.

Polarographic technique: Notes on ——. Jessop, 273.

***Polarography:** Applying cathode ray oscillograph to —— ; general lay-out and uses of cathode ray polarograph. Airey, 304.

*Applying cathode ray oscillograph to —— : underlying principles. Randles, 301.

*Four papers on ——. 291.

*Fundamental laws of ——. Heyrovský, 229.

Polyethylene glycols: Solid —— (Carbowax compounds). Quantitative detmn. in biological materials. Shaffer and Critchfield, 404.

Polymers: Detmng. hydroperoxides in rubber and synthetic ——. Laitinen and Nelson, 369.

Post-mortem specimens: Rapid qualitative and quantitative detmn. of barbiturates from ——. Valov, 256.

Potash: Rapid semi-micro volumetric detmn. of —— in leaf ash. Chapman, 374.

Potassium: Detmng. sodium and —— in silicates. Kallmann, 167.

Routine analysis of sodium- —— alloys. Walters and Miller, 317.

Potassium cobaltinitrite: Explosion in detmng. cobalt as ——. Broughton, Laing and Wentworth, 410.

Potassium ethyl xanthogenate: Micro-separation of zinc and aluminium by means of ——. Wenger, Abramson and Besso, 76.

Potassium hydroxide: Rare-earth metal oxides. III. Pptn. from —— melts. Marsh, 169.

Powder(s): Detmng. aromatic amines and substituted ureas in smokeless ——. Volumetric bromination procedure. Waugh, Harbottle and Noyes, 73.

Prepng. standard —— for reference in particle-size measurement. Gooden and Updike, jun., 270.

Powdered materials: Prepng. —— for electron microscopy. Schuster and Fullam, 224.

Precipitation reactions: Sensitivity of —— in dilute electrolytes. McAlpine, 116.

Precision: Improving —— by repeated measurements. Application to analytical control methods. Mandel, 324.

Preserves Order, 1947. 398.

Pressure: Precise low- —— measurements with thermocouple gauge. Smiley, 497.

Procaine hydrochloride: Microscopical identification of butacaine sulphate (Butyn) and —— (Novocaine). Keenan, 547.

Production control: Applying polarographic analysis to —— in non-ferrous metallurgy. Pletenev, Arefyeva, Tal and Dubovitzkaja, 415.

***Propellant explosives:** Estmng. volatile matter content of ——. 3. Estmng. acetone. Bonner, 434.

*Estmng. volatile matter content of ——. 2. Estmng. ethyl alcohol and ether. Bonner, 47.

1-n-Propoxy-2-amino-4-nitrobenzene: Detmng. the sugar substitute ——. Hoeke, 548.

Protein(s): Application of partition chromatography to estmng. monoamino acids in ——. Tristram, 260.

Applying Sendroy's iodimetric chloride titration to —— -containing fluids. Van Slyke and Hiller, 364.

Colorimetric estmn. of methionine in —— and foods. Horn, Jones and Blum, 210.

Colorimetric estmn. of —— in body fluids. Albanese, Irby and Saur, 208.

Estmng. threonine and serine in ——. Rees, 310.

Hydrolysis procedures for estmng. tryptophan in —— and foodstuffs by microbiological procedure. Greenhut, Schweigert and Elvehjem, 25.

Microbiological estmn. of methionine in —— and foods. Horn, Jones and Blum, 211.

Microbiological estmn. of methionine in —— and foodstuffs. Riesen, Schweigert and Elvehjem, 69.

Spectrophotometric detmn. of tyrosine and tryptophan in ——. Goodwin and Morton, 260.

Proteus HX19: Microbiological estmn. of nicotinic acid using ——. Grossowicz and Sherstinsky, 362.

***Provitamin-A :** Chromatographic adsorption method for estmng. —— -content of foodstuffs. Ramasarma, Hakim and Rao, 194.

***Pterins:** Applications of fluorimetric analysis to study of ——. Simpson, 382.

Puerto Rican fatty oils. VI. Characteristics and composition of "Molinillo" seed oil. Asenjo, Goyco and Martinez-Pico, 485.

Pyrethrin(s): Detmng. —— content of dilute preparations of pyrethrum flowers in oil. Bray and Lord, 264.

Detmng. —— in pyrethrum concentrates in mineral oil. Martin and Brightwell, 458.

Pyrethrum: Ditto, ditto. Martin and Brightwell, 458.

flowers : Detmng. pyrethrin content of dilute preparations of —— in oil. Bray and Lord, 264.

Pyridine nucleotides: Rapid fluorimetric estmn. of total —— in red blood cells. Levitas, Robinson, Rosen, Huff and Perlzweig, 363.

Pyridoxal: Colorimetric detmn. of pyridoxine, —— and pyridoxamine. Ormsby, Fisher and Schlenk, 260.

Pyridoxamine: Ditto, ditto. Ormsby, Fisher and Schlenk, 260.

Pyridoxine: Ditto, ditto. Ormsby, Fisher and Schlenk, 260

Pyrophosphoric acid in detmng. nickel in cast iron and steel. Kononiuk, 413.

Q

Qualitative analysis: System of —— for common metals in presence of phosphates, using ammonium benzoate. Austin, 443.

Qualitative elementary analysis of organic substances by hydrogenation. Slooff, 163.

Quantitative organische Mikroanalyse. Pregl. 5th Edn. (Review), 461.

Quartz: Detmng. —— content of clay fraction of soils. de Leenheer and Waegemans, 217.

***Quenching:** Fluorescence —— in solutions. Bowen, 379.

Quinaldic acid: Detmng. copper in cast iron and steels with ——. Flagg and Vanas, 121.

Quinine: Titrimetric detmn. of di-acidic bases (nicotine, ——) in mixtures with mono-acidic bases, and simplified assay for nicotine. Trautner and Neufeld, 160.

Quinone oximes: Chromatographic resolution of —. Gullstrom, Burchfield and Judy, 77.

R

***Radiant heater:** Evaporation from beakers with ——. Howes, 111.

Radioactive studies. Analytical method for detmng. long-life carbon ^{14}C. Henriques and Margnetti 80.
  Analytical procedure for measurement of long-lived radioactive sulphur ^{35}S, with Lauritzen electroscope, and comparison of electroscope with special Geiger counter. Henriques, Kistiakowsky, Margnetti and Schneider, 80.
  Analytical procedure for measurement of radioactive arsenic of 90-day half-life. Henriques and Margnetti, 80.
  Utilisation of radioactive isotope dilution procedures for special types of chemical problems. Detmng. the three individual components of mixtures of dibenzyl-sulphide, -sulphoxide and -sulphone. Henriques and Margnetti, 80.

Raman spectra: Quantitative analysis of hydrocarbon mixtures by means of ——. Rosenbaum, Martin and Lauer, 261.

Rare earth(s): Analytical chemistry of —— Active-oxygen detmn. Barthauer and Pearce, 217.
  Detcng. ytterbium in —— mixtures. Beck, 124.
  metal oxides. III. Pptn. from potassium hydroxide melts. Marsh, 169.
  metal oxides. II. Study of oxide pptn. by nitrate fusion. Marsh, 169.
  New principle of fractionation of —— with nitrilo-triacetate Separating lanthanum and cerium. Beck, 74.
  Separating —— elements by chloride volatilisation. Vickery, 414.

Raymond method: Colorimetric assay of digitoxin by modified ——. Anderson and Chen, 362.

Red blood cells: Rapid fluorimetric estmn. of total pyridine nucleotides in ——. Levitas, Robinson, Rosen, Huff and Perlzweig, 363.

Reducing gases: Detmng. rates of de-oxidation of iron oxide materials in ——. Barrett and Wood, 318.

***Reducing groups:** Chapman and McFarlane method of estmng. —— in milk powder. Lea, 336.

Reducing sugars: Electrometric estmn. of sucrose and ——. Coalstad, 409.

Reductor: Behaviour of uranyl solutions in mercury ——. Caley and Rogers, 216.

Refractometric analysis: Characterisation of pure hydrocarbons. —— as key to evaluation of structure. Wendland, 114.

Reinecke's salt: Detmng. narceine in narceine sodium salicylate with ——. van Arkel and Kramer, 547.

Repeated measurements: Improving precision by ——. Application to analytical control methods. Mandel, 324.

Resin(s): Analysing dilute melamine —— solutions. Ivarsson and Steenberg, 32.
  Chromatographic adsorption of amino acids on organic exchange ——. Cleaver, Hardy and Cassidy, 450.
  Ion exchange —— in study of chrome liquors. Adams, 169.

Riboflavine: Chemical estmn. of ——. Scott, Hill, Norris and Heuser, 24.
  Fluorimetric estmn. of —— in urine. Morell and Slater, 257.
  Microbiological estmn. of —— in blood. Bradford and Coke, 24.
  Modification of fluorimetric method of estmng. —— in biological materials. Slater and Morell, 257.

Rocks: Detmng. gallium in silicate ——. Sandell, 457.

Rubber: Detmng. hydroperoxides in —— and synthetic polymers. Laitinen and Nelson, 369.

S

Salicylaldehyde reaction: Specificity of —— for detcng. acetone. Thomson, 28.

Sampling: Most economical —— for chemical analysis. Churchman, 323.

Sand(s): Adsorption of aneurine on ——. Ridyard, 68.
  Effect of sieve loading on results of sieve analysis of natural ——. Shergold, 126.

Sauces: Pickles and —— Order, 1947. 309.

Scharer phosphatase test: Modified —— for milk and soft cheeses. Horwitz, 544.

Sea water: Rapid electrometric detmn. of alkalinity of ——. Anderson and Robinson, 375.

***Seals:** Analysing nickel - cobalt - iron alloys used in glass-to-metal ——. Chirnside, Cluley and Proffitt, 351.

Seed(s): Microscopical differentiation of common mustard —— with polarised light. Keenan, 448.
  oils of Sudan Caesalpinioideae. Grindley, 111.
  oil: Puerto Rican fatty oils. VI. Characteristics and composition of "Molinilla" seed oil. Asenjo, Goyco and Martinez-Pico, 485.

Selenium compounds: Analysing organo- ——. McCullough, Campbell and Krilanovich, 30.

Semi-quantitative inorganic analysis: Method of ——. Wenger, Monnier and Piguet, 116.

Sendroy: Applying ——'s iodimetric chloride titration to protein-containing fluids. Van Slyke and Hiller, 364.

Sensitivity of precipitation reactions in dilute electrolytes. McAlpine, 116.

Serine: Estmng. threonine and —— in proteins. Rees, 310.
  Micro-detmn. of —— and ethanolamine in phospholipid hydrolysates. Burmaster, 23.

Serum: Detmng. —— phosphate by molybdivanadate method. Simonsen, Wertman, Westover and Mehl, 312.
  Estmng. penicillin in ——. Use of glucose, phenol red and —— water. Fleming and Smith, 548.
  Estmng. vitamin A and carotene in small quantities of blood ——. Bessey, Lowry, Brock and Lopez, 161.
  Photometric detmn. of total cholesterol in plasma or —— by modified Liebermann-Burchard reaction. Saifer and Kammerer, 27.
Sheet materials: Isostatic method for detmng. gas permeability of ——. Davis, 81.
Shrimp: Indole in ——. Duggan and Strasburger, 545.
Sieve loading: Effect of —— on results of —— analysis of natural sands. Shergold, 126.
Silicate(s): Detmng. sodium and potassium in ——. Kallmann, 167.
  rocks : Detmng. gallium in ——. Sandell, 457.
Silicon: Absorptiometric detmn. of —— in steels. Gentry and Sherrington, 121.
  Detmng. —— in relatively non-volatile organo- —— compounds. Gilman, Clark, Wiley and Diehl, 264.
Silver nitrate: Amperometric titration of cyanide with ——, using rotating platinum electrode. Laitinen, Jennings and Parks, 319.
Silver sulphide method for detmng. cadmium. Lapin, 373.
Skeletal muscle: Tocopherol content of ——. Comparison of chemical and bio-assay methods. Kaunitz and Beaver, 161.
Slags: Polarographic detmn. of lead in lead ——. Fainberg and Tal, 268.
Smokeless powder: Detmng. aromatic amines and substituted ureas in ——. Volumetric bromination procedure. Waugh, Harbottle and Noyes, 73.
Soap: Detmng. total hardness of water by means of —— solution. Teterkin, 375.
  *film method : Apparatus for gas analysis by ——. Gooderham, 520.
***Society of Public Analysts:** Address of Retiring President. Monier-Williams, 137.
  Anniversary Dinner, 1947. 129.
  Annual General Meeting, 1947. 129.
  Annual General Meeting of Biological Methods Group, 83.
  Annual General Meeting of Microchemistry Group, 177.
  Annual Report of Council, 1947. 131.
  Presentation of Address to Chemical Society, 327.
  Second Annual General Meeting of Physical Methods Group, 35.
  Third Annual General Meeting of Physical Methods Group, 503.
  Twelfth Annual General Meeting of Scottish Section, 177.
  Twenty-second Annual General Meeting of North of England Section, 83.
Sodium: Detmng. —— and potassium in silicates. Kallman, 167.
  Routine analysis of —— -potassium alloys. Walters and Miller, 317.
  Simplified detmn. of —— in biological fluids. Bradbury, 112.
***Sodium carbonate:** Factors affecting —— fusion of beryl. Osborn, 475.
***Sodium dichromate:** Detmng. sulphate in ——. Wylie, 250.
Sodium fluoride: Detmng. nickel and cobalt in iron ores with preliminary removal of iron by means of ——. Tananaev and Silnitschenko, 373.
Sodium oxalate: Cacotheline in volumetric analysis. I. Detmng. calcium salts by titration with ——. Kutschment and Gengrinovitsch, 492.
Sodium thiosulphate: Separating copper and zinc with —— in analysing brass. Sarudi (v. Stetina), 410.
Soft Drinks (Amendment) Order, 1947. 153; (Amendment No. 2) Order. 398 ; (Amendment No. 3) Order. 399.
Soil(s): Detmng. quartz content of clay fraction of ——. de Leenheer and Waegemans, 217.
  Identifying and estmng. montmorillonite group of minerals, with special reference to —— clays. MacEwan, 218.
  Morgan method of —— testing. IV. Use of Spekker absorptiometer for estmng. phosphate. Tinsley and Pizer, 124.
Soup: Meat Products, Canned Meat and Canned —— (Control and Max. Prices) (Amendment No. 3) Order, 1946. 64.
South African fish products: XXII. Periodic acid as reagent in analysing unsaponifiable fraction of natural fats. Karnovsky and Rapson, 22.
Specific gravity: Application of differential manometer to measurement of fluid density and —— with special reference to temperature effects. Cowherd, 80.
  Detmng. —— of extracts of jams, syrups, etc. by means of density gradient. van der Kamer and Jansen, 399.
Spectral transmission: Particle size by ——. Bailey, 222.
Spectrochemical analysis: Errors in —— (flame analysis). Oertel and Stace, 497.
  Flame method of ——. Roach, 174.
  Index to the Literature on ——, Part II, 1940 to 1945. Scribner and Meggers. (Review), 325.
  Purifying carbon rods for ——. I. Simple apparatus for purifying by resistance heating. Hoogland. II. Carbon rods for —— of solutions. Schmidt. III. Deinum, 419.
Spectrographic analysis: Internal standard method of —— as applied to detmng. lead in high-purity zinc. Griffith and Kirkbride, 223.
Spectrography: Standardised Substances for ——, Chemical Analysis and Research. Johnson, Matthey and Co., Ltd., 254.
Spectrophotometric changes during oxidation of vitamin A oils. Halpern, 78.
Spekker absorptiometer: Morgan method of soil testing. IV. Use of —— for estmng. phosphate. Tinsley and Pizer, 124.
Spot tests: Chromatographic ——. Hopf, 556.
  *for detcng. alloying elements in lead-base alloys. Evans and Higgs, 105.
  *for detcng. alloying elements in tin-base alloys. Evans and Higgs, 439.
  *for detcng. alloying elements in zinc-base alloys. Evans and Higgs, 101.
Standard powders: Prepng. —— for reference in particle-size measurement. Gooden and Updike, jun., 270.
Standardised Substances for Spectrography, Chemical Analysis and Research. Johnson, Matthey and Co., Ltd., 254.
Stannous chloride: Cacotheline in volumetric analysis. II. Detmng. tervalent iron by titration with —— solution. Kutschment and Gengrinovitsch, 492.

***Starch**(es)-iodide complex: Photometric detmn. of dissolved oxygen in condensates and feed waters by means of ——. Bairstow, Francis and Wyatt, 340.
  Recording viscometer for ——. Kesler and Bechtel, 407.
***Statistical background** in bio-assay. Fieller, 37.
Steel(s): Absorptiometric detmn. of silicon in ——. Gentry and Sherrington, 121.
  Detmng. aluminium in high alloy ——. Fogelson and Kalmikova, 493.
  Detmng. copper in cast iron and —— with quinaldic acid. Flagg and Vanas, 121.
  Detmng. hydrogen in liquid ——. Wells and Barraclough, 457.
  Detmng. nickel in cobalt —— and alloys. Kirtchik, 553.
  Detmng. oxygen in iron and ——. Vigna, 456.
  Detmng. phosphorus in austenitic chromium-nickel ——. Methods of analysis of Metallurgy Division of B.I.S.R.A., 412.
  *Detmng. tungsten in ——, using toluene-3:4-dithiol. Removal of molybdenum interference by selective extraction. Bagshawe and Truman, 189.
  *Microchemical detmn. of molybdenum in ——. Wells and Pemberton, 185.
  Phenylanthranilic acid in volumetric detmn. of molybdenum in —— and ferro-molybdenum. Fogelson and Kalmikova, 413.
  Pyrophosphoric acid in detmng. nickel in cast iron and ——. Kononiuk, 413.
  Spectro-analytical detmn. of boron in ——. Gegetschkori and Falkova, 411.
Steroid(s): Colorimetric estmn. of reducing ——. Heard and Sobel, 71.
  hormones: Polarographic estmn. of ——. Polarography of neutral 17-ketosteroids in urinary extracts. Barnett, Henly and Morris, 26.
Still: Simple laboratory falling film molecular ——. Farmer and Sutton, 174.
Streptomycin: Polarographic analysis of ——. Levy, Schwed and Sackett, 310. Erratum, 449.
Strontium: Quantitative separation of barium from ——. Beyer and Reiman, 551.
Sublimation apparatus: Simple ——. Pitha, 126.
Sucrose: Electrometric estmn. of —— and reducing sugars. Coalstad, 409.
Sudan Caesalpinioideae: Seed oils of some ——. Grindley, 111.
Sugar(s): Chromatography of —— and their derivatives. Georges, Bower and Wolfrom, 316.
  Electrometric estmn. of sucrose and reducing ——. Coalstad, 409.
  Rapid photocolorimetric micro-procedure for blood ——. Polis and Sortwell, 162.
  Raw ——, sampling, mixing and testing. Kenney, 402.
  substitute: Detmng. ——, 1-*n*-propoxy-2-amino-4-nitrobenzene. Hoeke, 548.
Sulpha drugs: Detmng. sulphathiazole and other —— in honey. Shepard and Fisher, 448.
***Sulphate:** Detmng. —— in sodium dichromate. Wylie, 250.
  Rapid detmn. of —— impurities in cryolite. Karjakin and Muraschova, 266.
Sulphathiazole: Detmng. —— and other sulpha drugs in honey. Shepard and Fisher, 448.
***Sulphide** precipitation: Indicator changing at *p*H 0·5 for control of ——. Andrew, 481.
Sulphite: Detmng. dissolved oxygen in presence of ——. Janssen, 496.
Sulphonamides: Detmng. —— in tungstic acid (Folin-Wu) blood filtrates. Schmidt, 25.
  Identifying ——. Witte, 546.
***Sulphoxide group:** Reductometric detmn. of —— and amine oxide group. Glynn, 248.
Sulphur compounds: Identifying —— in gas mixtures. Oldach and Field, 219.
  Conversion of organic —— to hydrogen sulphide for analysis. Field and Oldach, 115.
  Detmng. oxides of —— in flue gas. Gillham, 321.
  Entrainment of cobalt and —— in iron separations. Pinkney, Dick and Young, 122.
  Radioactive studies. Analytical procedure for measurement of long-lived —— ^{35}S, with Lauritzen electroscope, and comparison of electroscope with special Geiger counter. Henriques, Kistiakowsky, Margnetti and Schneider, 80.
Sulphur trioxide: Automatic detmn. of —— in flue gas. Gillham, 322.
Sulphuric acid: Analysing mixtures of mercurous and mercuric mercury and ——. Warshowsky and Elving, 493.
  Improved analysis of fuming and concentrated —— by water titration. Brand, 168.
  Nitration in ——. III. Influence of nitric acid and other substances on oleum-water titration. Brand, 169.
Supply of laboratory apparatus and chemicals. 176, 227, 326, 376.
Surface-active agents: Qualitative method for detcng. ——. Hoyt, 270.
Synthetic polymers: Detmng. hydroperoxides in rubber and ——. Laitinen and Nelson, 369.
Syrups: Detmng. specific gravity of extracts of jams, ——, etc., based on density gradient. van der Kamer and Jansen, 399.

T

Tankage: Haemoglobin in meat scraps and ——. Reiser, 446.
Tantalum: Separating niobium and —— from titanium. Oschman, 373.
T.A.P.P.I.: Cellulose in wood. —— Tentative Standard T17 m-46. 366.
Tartrate(s): Metallic complexes of —— and citrates; structure and behaviour in dilute solutions. I. Cupric and nickelous complexes. Bobtelsky and Jordan, 213.
  Polarographic investigations of oxalate, citrate, and —— complexes of ferric and ferrous iron. Lingane, 370.
Tetra-ethyl lead: Detmng. —— in gasoline by X-ray absorption. Sullivan and Friedman, 269.
  Micro-detmn. of —— in gasoline. Gordon and Burdett, 454.
Thallium: Combined micro-gravimetric and titrimetric micro-detmn. of —— in toxicological material. Reith and Gerritsma, 221.
Thermocouple gauge: Precise low-pressure measurements with ——. Smiley, 497.
***Thimble:** Micro filter ——. Bowden, 542.
Thiobarbituric acid: Estmng. barbituric and —— in biological materials. Raventos, 487.
Thiocyanate: Colorimetric detmn. of molybdenum as —— complex. Tananaev and Lochvitzkaja, 455.
  complex as means of extracting cobalt before micro-detmn. by other methods. Bayliss and Pickering, 222.
  ions: Semimicro detcn. of —— and halide ions. Porter, 76.

Thiocyanate—*continued*
  *Stability of cobaltous —— complex in ethyl alcohol - water mixtures and photometric detmn. of cobalt. Uri, 478.
Thorpe's Dictionary of Applied Chemistry. 4th Edn. Vol. VIII. Meth - Oils, Essential. (Review), 499.
Threonine: Estmng. —— and serine in proteins. Rees, 310.
Tin: Applying polarographic analysis to production control in non-ferrous metallurgy. III. Detmng. copper, bismuth, lead, cadmium and zinc impurities in —— and lead - —— solders. Pletenev, Arefyeva, Tal and Dubovitzkaja, 416.
  Polarographic detmn. of —— in foods and biological materials. Godar and Alexander, 111.
  *Polarographic detmn. of ——, lead and zinc in phenol. Wilson and Hutchinson, 149.
***Tin-base alloys:** Spot-tests for detcng. alloying elements in ——. Evans and Higgs, 439.
Titanium: Colorimetric detmn. of —— with disodium-1:2-dihydroxy-benzene-3:5-disulphonate. Yoe and Armstrong, 552.
  Separating niobium and tantalum from ——. Oschman, 373.
***Titration:** Amperometric ——. Stock, 291.
Tocopherol: Colorimetric estmn. of ——. Emmerie, 209.
  content of skeletal muscle ; comparison of chemical and bio-assay methods. Kaunitz and Beaver, 161.
***Toluene-3 : 4-dithiol:** Detmng. tungsten in steel, using ——. Removal of molybdenum interference by selective extraction. Bagshawe and Truman, 189.
Toxicological material: Combined micro-gravimetric and titrimetric micro-detmn. of thallium in ——. Reith and Gerritsma, 221.
***Trans-esterification:** Detmng. acetyl groups by ——. Stuart, 235.
Triethyl phosphate: Fractional separation of hafnium and zirconium by means of ——. Willard and Freund, 216.
Tryptophan: Hydrolysis procedures for estmng. —— in proteins and foodstuffs by microbiological procedure. Greenhut, Schweigert and Elvehjem, 25.
  Micro-Kjeldahl detmn. of nitrogen in gramicidin and ——. Comparison of Gunning-Arnold-Dyer and Friedrich methods. White and Secor, 263.
  Spectrophotometric detmn. of tyrosine and —— in proteins. Goodwin and Morton, 260.
Tswett adsorption columns: Conditions affecting the sequence of organic compounds in ——. Strain, 77.
***d-Tubocurarine chloride:** Assay of solutions of —— for injection. Foster, 62.
Tungsten: Bivalent chromium solution for volumetric detmn. of ——. Detmng. —— in —— concentrates and ferro- ——. Tschernichov and Gorjuschina, 371.
  *Detmng. —— in steel, using toluene-3 : 4-dithiol. Removal of molybdenum interference by selective extraction. Bagshawe and Truman, 189.
  *Direct colorimetric detmn. of —— in cast iron. Westwood and Mayer, 464.
  Quantitative detmn. of ——, molybdenum, and vanadium alone and in presence of ferric iron, with 8-hydroxyquinoline. Niericker and Treadwell, 214.
Tungstic acid blood filtrates: Detmng. sulphonamides in —— (Folin-Wu). Schmidt, 25.
Tyrosine: Spectrophotometric detmn. of —— and tryptophan in proteins. Goodwin and Morton, 260.

U

Uranium: Detmng. —— with 8-hydroxyquinoline. Claassen and Visser, 123.
Uranyl solutions: Behaviour of —— in mercury reductor. Caley and Rogers, 216.
***Urate:** zinc —— oxidation reaction, selective test for zinc. Fearon, 541.
Ureas: Detmng. aromatic amines and substituted —— in smokeless powder. Volumetric bromination procedure. Waugh, Harbottle and Noyes, 73.
Urinary extracts: Polarographic estmn. of steroid hormones. Polarography of neutral 17-ketosteroids in ——. Barnett, Henly and Morris, 26.
***Urine:** Alcohol in blood and ——. Andrew and Neubauer, 21.
  Detmng. N′-methylnicotinamide in ——. Huff and Perlzweig, 363.
  *Estmng. traces of mercury in ——. Milton and Hoskins, 6.
  Fluorimetric estmn. of riboflavine in ——. Morell and Slater, 257.
  Iodimetric estmn. of glucose in —— and blood. Noyons, 210.
  Polarographic detmn. of oestrone in ——. Bjornson and Ottesen, 403.

V

Vanadium: Extracting —— from aqueous solutions by *iso*propyl ether. Lingane and Meites, jun., 216.
  Quantitative detmn. of tungsten, molybdenum, and ——, alone and in presence of ferric iron, with 8-hydroxyquinoline. Niericker and Treadwell, 214.
Vanillin: Microchemical detcn. of —— and bourbonal. Rutten, 449.
Varnishes: Volumetric detmn. of water in paints and ——. Swann, 170.
Vegetable(s): Canned Fruit and —— (Amendment No. 2) Order, 1947. 543.
  Detmng. DDT deposits on fruits, —— and vegetation. Carter and Hubanks, 4[illegible].
  material : Detmng. traces of copper in —— with diethyldithiocarbamate. Reith, Rameau, Mulder, Seekles, Kniphorst and Deijs, 555.
Vegetation: Detmng. DDT deposits on fruits, vegetables and ——. Carter and Hubanks, 401.
Viscometer: Recording —— for starches. Kelser and Bechtel, 407.
Viscose: Disintegration of —— in analysing paper fibres. Browning and Graff, 491.
***Vitamin:** Applications of fluorimetry in —— analysis. Kodicek, 385.
Vitamin A: Activated glycerol dichlorohydrin. Colorimetric reagent for ——. Sobel and Werbin, 27.
  *Chromatographic adsorption method for estmng. pro- —— content of foodstuffs. Ramasarma, Hakim and Rao, 194.

Vitamin A—*continued*
- Estmng. —— and carotene in small quantities of blood serum. Bessey, Lowry, Brock and Lopez, 161.
- Spectrophotometric changes during oxidation of —— oils. Halpern, 78.

***Vitamin C:** Oxalic acid extractant in —— assay. Wokes, 63.

Vitamin D: Colorimetric estmn. of ——, based on formation of carbenium salts. Schaltegger, 70.

***Volatile matter:** Estmng. —— content of propellant explosives. 3. Estmng. acetone. Bonner, 434.
- *Estmng. —— content of propellant explosives. 2. Estmng. ethyl alcohol and ether. Bonner, 47.

Volatilisation: Separating rare-earth elements by chloride ——. Vickery, 414.

W

Water(s): Analysing furfural- —— solutions. Griswald, Klecka and West, 165.
- Colorimetric detmn. of hardness of ——. Dratschev, 460.
- Current Waterworks Practice. Practical Treatise on the Provision of —— for Urban and Rural Communities. Maxwell. (Review), 270.
- *Detmng. nitrates in ——. Allerton, 349.
- Detmng. total hardness of —— by means of soap solution. Teterkin, 375.
- New simple titration methods for detmng. hardness of ——. Schwarzenbach, Biedermann and Bangerter, 322.
- *Photometric detmn. of dissolved oxygen in condensates and feed —— by means of starch-iodide complex. Bairstow, Francis and Wyatt, 340.
- Rapid electrometric detmn. of alkalinity of sea ——. Anderson and Robinson, 375.
- *Stability of cobaltous thiocyanate complex in ethyl alcohol - —— mixtures and photometric detmn. of cobalt. Uri, 478.
- titration: Improved analysis of fuming and concentrated sulphuric acid by ——. Brand, 168.
- titration: Nitration in sulphuric acid. III. Influence of nitric acid and other substances on oleum- ——. Brand, 169.
- Volumetric detmn. of —— in paints and varnishes. Swann, 170.

Waterworks: Current —— Practice. Practical Treatise on the Provision of Water Supplies for Urban and Rural Communities. Maxwell. (Review), 270.

Weights: Factors affecting constancy of analytical ——. Craig, 420.

White blood cells: Estmng. glycogen in whole blood and ——. Wagner, 162.

***Wines:** Formation of racemic calcium tartrate in ——. Cambitzi, 542.

Wood: Cellulose in ——. T.A.P.P.I. Tentative Standard T17 m-46. 366.

X

X-ray analysis: Identifying crystalline organic compounds by ——. de Lange and Houtman, 406.

X-ray diffraction powder patterns: Identifying organic compounds by use of chromium target ——. Matthews and Michell, 174.

X-ray identification and crystallography of aldehydes and ketones as 2:4-dinitrophenylhydrazones. Clark, Kaye and Parks, 407.

Y

Yeast microbiological method for estmng. nicotinic acid. Williams, 312.

Ytterbium: Detcng. —— in rare-earth mixtures. Beck, 124.

Z

Zinc: Applying polarographic analysis to production control in non-ferrous metallurgy. I. Detmng. copper, lead and —— impurities in metallic cadmium. Pletenev, Arefyeva, Tal and Dubovitzkaja, 415.
- Ditto. III. Detmng. copper, bismuth, lead, cadmium and —— impurities in metallic tin and lead - tin solders. Pletenev, Arefyeva, Tal and Dubovitzkaja, 416.
- Ditto. V. Rapid detmn. of lead, cadmium and —— in ores and refuse from ore-enrichment (——, lead and cadmium ores). Pletenev, Arefyeva, Tal and Dubovitzkaja, 417.
- Internal standard method of spectrographic analysis as applied to detmng. lead in high-purity ——. Griffith and Kirkbride, 223.
- Micro-separation of —— and aluminium by means of potassium ethyl xanthogenate. Wenger, Abramson and Besso, 76.
- *Polarographic detmn. of tin, lead and —— in phenol. Wilson and Hutchinson, 149.
- *Pptng. aluminium and separating it from cobalt, chromium, nickel or —— by means of ammonium benzoate. Smales, 14.
- Separating copper and —— with sodium thiosulphate in analysing brass. Sarudi (v. Stetina), 410.
- *urate oxidation reaction, selective test for ——. Fearon, 541.

***Zinc-base alloys:** Spot-tests for detcng. alloying elements in ——. Evans and Higgs, 101.

***Zinc urate** oxidation reaction, selective test for zinc. Fearon, 541.

Zirconium: Fractional separation of hafnium and —— by means of triethyl phosphate. Willard and Freund, 216.

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